

The commercialization of space

The US Administration is planning to privatize its weather and remote sensing satellites. The facts suggest it should think again and concentrate on better ways to increase the private stake in space.

THE Reagan Administration seems obstinately determined to ignore expert advice and to press ahead with a plan to sell the government remote sensing and weather satellites to private business. In November, the Department of Commerce will invite bids for the purchase of all or any of the Landsat satellites, the geostationary and polar weather satellites and their associated ground stations and data processing centres. The pity is that by trying, wrongly, to commercialize an inappropriate system, the administration will simply discredit the good idea of increasing commercial investment in space systems that can really be made to pay for themselves.

Congress and the Department of Commerce have been at loggerheads over the proposed sale for months. The department did its cause little good by permitting a whiff of scandal to taint its early discussions with potential satellite buyers. But the real argument against going ahead with the administration's plan is that, in this instance, privatization will not achieve its main purpose: reduction of costs through the free play of market competition.

The reason is that the market for remote sensing data is not yet big enough to generate revenues equal to the cost of operating the Landsat system. According to the Office of Technology Assessment, the current US market for Landsat data is worth approximately \$10 million a year compared with operating and maintenance costs in 1983 of about \$23 million. The remote sensing of land from space has attracted a small number of enthusiastic customers who use the data for research or land resource management, but the principal user is the government itself. Given the infancy of the market, and Landsat's design limitations (it is considerably less capable than the French SPOT will be), a successful transfer to the private sector will require at least a decade of government subsidy.

The administration tacitly recognized as much when it decided to consider including the weather satellites in a Landsat sale. A company willing to take on both systems could offset its losses on Landsat against the guaranteed sale of meteorological data to the weather satellites' original owner, the US Government. It is an outcome that would be as lucrative for the companies involved as it would be expensive for the taxpayer. There is little market for basic meteorological information outside the government and none is likely to develop except in the unimaginable event of the government deciding to charge citizens for their weather information. Even then, it is hard to see how a proprietary approach to such data could then be reconciled with the United States' international meteorological obligations and its obligation to protect the life and property of private citizens.

Its inability to earn money is not the only problem facing a potential owner of Landsat. An owner would have to find some way to maintain US undertakings to make Landsat data available to Third World countries that request it, undertakings that are already regarded with considerable suspicion by some of the countries over which Landsat orbits. The Department of Defense has concerns of its own. It is reportedly insisting that it retains the right to monitor the kind of sensors mounted on the Landsat system lest a private operator detect objects the Department of Defense prefers to keep secret. The Pentagon also wants the system to revert to government control in the event of a national crisis.

Sadly, the plan to sell Landsat and the weather satellites has

distracted attention from the real and beneficial possibilities of a major increase in the private sector stake in space. The recent successes of the space shuttle and Europe's Ariane may mark the beginning of a period in which healthy competition brings down the price of placing satellites in orbit and makes the prospects of space-based research and manufacturing on a large scale more realistic. It would be wrong to expect this new and more benign space race to conform to the laws of a real market, since all the systems involved are subsidized at different levels. But growing commercial interest in what can be done in space does at least hold out the promise of some financial return for the large investment the space agencies have made in their respective systems.

One caveat should be entered, however. Right as it is for the National Aeronautics and Space Administration to encourage private companies to exploit the potential of the shuttle, it would be wrong, in a tactical sense, to exaggerate what can be achieved industrially in space. Johnson and Johnson and the McDonnell Douglas Corporation have initiated interesting experiments to manufacture drugs in space by means of electrophoresis. These and other experiments are tightly constrained, however, by the fact that only extremely expensive products could justify the high cost per weight of space-based manufacturing. The shuttle was originally conceived as part of a versatile Space Transportation System capable of performing a large variety of missions. To overstate its commercial prospects would be to court disappointment, and to jeopardize its use as a research vehicle and a building block on which new government-funded ventures could be based.

Acid rain, political bile

The lack of scientific solution should temper the political response to acid rain in Europe.

THE most complicated issues in science have a habit of being the most political. One such issue is "acid rain", a term which conjures up a witches' brew of uncertain science and political posturing. The fundamental ingredient of the brew is doubt. Politics breeds on doubt. Science, which aims to remove doubt, is therefore the antithesis of politics: its objective must be to make politicians redundant.

That must be the goal of those scientists in West Germany who have now clubbed together to investigate the decay of the Black Forest (see page 742), whatever pressures they may find themselves under from their ministries of research, agriculture and the interior. For in Germany, as in Scandinavia, there is a chance that internal costs could be externalized: the polluter should pay, and the polluter may not, in the main, be German or Scandinavian but from further west in Europe. This is an apparently fair principle (though it may be considered an unfair act of God that the wind blows mostly from the west). Its consequence, for scientists, is that those who are German and Scandinavian will be encouraged to "prove" the bad effects of acid rain while, say, French and British scientists will be encouraged to do the reverse. All must, of course, resist these pressures and no doubt they would like to: but in the case of the mid-European forests there is no time. Decisions will have to be taken before the scientific verdict is in. There lies the political rub. Scientific understanding of acid rain is still forming and unsure. It is at the



stage where science is process, not product and where, if scientists are forced to pass opinion, external factors play a part — matters of personality and cultural background, for example, and where is the next grant going to come from?

From the politician's point of view, this is mere detail: he or she is going to be provided with more or less biased advice, as usual. A decision in, say, the Council of Ministers of the European Community on sulphur dioxide emission controls will be like any other political decision, but should allow for one important fact. Where science is properly at work, doubt diminishes with time.

Diminishing doubt provides a genuine reason to accept an interim decision on, say, emission levels for airborne pollutants, in the knowledge that it can be corrected and improved as knowledge progresses. It is even possible to conceive of later compensation for early imbalances. Suppose, for example, West Germany managed to impose on France low sulphur dioxide levels at its factories, and that later it proved that oxides of nitrogen rather than of sulphur were the culprits. Why should West Germany not pay France a portion of the original costs of reducing emissions?

All of which might give the politicians pause for thought in their deliberations over a draft directive from the European Commission (COM(83) 173 of 8 April 1983). This proposes a uniform system for imposing emission limits on "stationary plant" (that is factories, not vehicles). Pushed by West Germany, the directive is moving fast. It was discussed by the Council of Ministers within what must be a record two months. The Council approved in principle that such a directive should exist. A small step but one that indicates that this environmental issue has reached the top of the European agenda. The next steps will be harder — particularly the ones that involve setting levels and hence costs. But they will be easier if it is understood that a maturing science, like the science of acid rain, enables later adjustment and so a sharing of risk as well as of costs. □

A cut too deep

Just as laser ranging proves its value to geophysicists, the British facility faces closure. It should be saved.

By coincidence this issue of *Nature* contains mixed blessings for those of its readers who take an interest in the solid body of our planet. The good news is that by using laser-ranging to track the minute changes in the orbit of the satellite Lageos, US scientists have been able to measure the rate at which the Earth's oblateness is decreasing (see p.757 and p.756). The decrease appears to be caused by the Earth's continuing internal readjustment following the end of the last ice age about 5,000 years ago; such measurements have wider ramifications because they enable the viscosity of material beneath the Earth's crust to be reduced, which in turn carries implications for convection in the mantle and for plate tectonics.

The bad news (see p.742) is that, in all likelihood, the UK Science and Engineering Research Council will have to stop supporting, among other worthwhile projects, the Satellite Laser Ranging unit at the Royal Greenwich Observatory in Sussex — the only facility in the country capable of carrying out such research. The Ranger seems set for extinction unless the council's expected budgetary problems for 1983/4 are alleviated from without, namely by the Treasury or the vagaries of the currency markets. As the facility has only recently been completed at a cost of £1 million or so, the situation seems farcical. But it is also informative, not only because it highlights a significant flaw in the current administration of British science but also because it promises to reveal something of the state of geophysics in the United Kingdom.

The flaw is that the Science and Engineering Research Council is subject to an accounting system which leaves forward planning, over the five- or even ten-year timescale needed for the development of a major project, at the mercy of short-term fluctuations in currency. As long as up to 20 per cent of its annual budget is spent on subscriptions to such international organizations as the European Space Agency and the European Organization for

Nuclear Research, it is inevitable that the council will be subject to such vicissitudes.

The system of government finance to which the council is currently subjected does not take this factor into account. At best it forces the council and its dependants to shift uneasily within the straitjacket of yearly financial accountability. At worst, as is happening this year, it forces the council to drastic and sometimes ludicrous measures, such as the virtual halt in further grant payments by the council's Astronomy, Space, and Radio Board until next financial year and the pending closure of the Satellite Laser Ranger. The hope is that such drastic consequences will concentrate the minds of the Treasury's consultative committee that is pondering the problem and upon whose recommendations the council is relying to avert yet more serious problems.

The fate of the Satellite Laser Ranger hangs, however, not only on the financial health of the Science and Engineering Research Council but also on the degree of commitment and support shown on its behalf by a community which — given the geophysical scope of interpretations of geodetic data — extends outside the council's dependants. Here the Natural Environment Research Council have a role to play. During the last financial year for which figures are available, it spent £44 million or so on solid-Earth geoscience. Of this, £5 million went towards geophysics. It should, therefore, at least be able to share with the Science and Engineering Research Council the £85,000 annual running costs of the Laser Ranger and so prevent the premature closure of a world-class facility. But both councils are reactive, rather than directive, bodies. Is there a sufficiently cohesive body of geophysicists within the United Kingdom that is energetic enough to ensure the Laser Ranger's survival? So far, apparently, not. □

JET set fair

Europe's nuclear fusion project is off the ground but has far to go.

THERE is a stark contrast between the smooth passage of development that culminated in the start up of the Joint European Torus last weekend (see p.746) and the dangerously bumpy path, often veering towards a cliff-edge, that was followed before the project was given a home.

Too expensive to be built by any single European nation but too important, for the political and scientific prowess of Europe, to be left to the Americans, the Soviets and the Japanese, nuclear fusion was an ideal project for the European Community right from the start. The problem was that national pride could not be placed wholly aside for the sake of the European ideal. Therefore, even when the decision had been taken to build the Joint European Torus, a prolonged period of political haggling was necessary before the rival nations conceded that it should be built in the United Kingdom. Most of the arguments against placing it there, some fairer than others, have been proven false by the completion of the five-year construction programme on time and at only a few per cent over cost.

Nevertheless, Europe's gain of a working torus does not bring the days of commercial nuclear fusion perceptibly closer. Not surprisingly the conditions of the start-up operation were even officially described as very modest. The first plasma was produced with a 60 kA current and lasted for one tenth of a second. The aim is to increase the current to 5,000 kA and to create a plasma for 10 seconds at a temperature of 100 million degrees. Formidable technical problems stand in the way.

Even then, the Joint European Torus is only an ignition demonstration device. If successful, it will be followed by a more advanced prototype before a demonstration reactor can be built. Those responsible for getting the Joint European Torus off to a flying start deserve a pat on the back, not least from the tax payers of Europe. But it is a sobering thought that neither those who have achieved the first step nor those who have paid for it are likely to be around to reap the benefits. The ever-receding projected date for the first commercial nuclear fusion power station is currently put at around 2030. □

India's nuclear power

Safety issue likely to override US export ban

Washington

THE United States is expected shortly to waive the provisions of the 1978 Nuclear Non-Proliferation Act and send India urgently-needed spare parts for its troubled American-built light-water reactors at Tarapur, north of Bombay. The State Department believes Congress will approve the shipment on humanitarian grounds, following reports that radiation leakage at the site has reached a hazardous level.

Although India's request for the parts will be a major issue during talks this month between Secretary of State George Shultz and Mrs Indira Gandhi, the Indian Prime Minister, Indian authorities have been trying to play down the problems at Tarapur. The Indian Atomic Energy Commission denies that radiation leakage has reached hazardous levels and the Indian Embassy in Washington refuses to confirm reports that one of the two General Electric reactors has been shut down and that the other is operating at below half power.

US congressional staff dismiss as "hyperbolic" claims in some Indian newspapers that conditions at Tarapur are so dangerous that local villagers have been recruited to work at the plant for only the very brief spells possible before they receive a dangerous lifetime dose of radiation. But staff aides believe that attempts by the Indian Government to stretch out dwindling fuel supplies by using low-quality fuel have resulted in serious contamination of the primary coolant.

The United States and India have been at loggerheads over the Tarapur plant ever since India's "peaceful" nuclear explosion in 1974 and the enactment of the Nuclear Non-Proliferation Act in 1978. The act obliges the United States to cut off enriched-uranium fuel exports until India agrees to place all its nuclear facilities under inspection by the International Atomic Energy Agency (IAEA). President Carter managed by a whisker to persuade Congress to authorize a fuel shipment in 1980, but the Reagan Administration subsequently arranged for the French to take over fuel supplies for Tarapur.

India maintains that its 1963 agreement on cooperation between the two countries at Tarapur ought not to have been suspended as a result of later domestic legislation in the United States. The two governments also disagree about the status of the agreement's original safeguards governing the use of plutonium produced at Tarapur. India argues that the safeguards, currently enforced by IAEA, lapse when the overall agreement expires in 1993. The United States maintains that the safeguards — which are already considered defective —

should continue to remain in force.

The history of discord surrounding Tarapur will complicate the administration's task of persuading Congress to agree to the shipment of spare parts. The House Foreign Affairs Committee recently rejected a proposal to amend the act so that nuclear components could be exported on health and safety grounds to nations which refused to accept IAEA supervision. Members felt it would be virtually impossible to distinguish between components necessary for health and safety and those needed for general operation of reactors.

Many congressmen are convinced that India misused US heavy water and Canadian uranium in order to achieve the 1974 explosion, and some are expected to argue

that India should be forced to solve its Tarapur problems by closing down the plants. Paul Leventhal, president of the Nuclear Control Institute, last week urged this course on Congress, arguing that the United States should not allow itself to be held responsible for the safety of the Indian nuclear programme. He added: "If India cannot operate the plant safely without the embargoed parts, it should either shut the plant down or make the non-proliferation commitments necessary to assure immediate shipment of the plants from the United States."

Even if a majority in Congress rejects this view, shipment of the necessary parts may not take place until the end of the year. If the administration seeks a waiver under the terms of the act, it must allow Congress 60 legislative days to decide whether to give its approval. An easier option would be to persuade a third country, such as Japan, to provide the General Electric-type components. But State Department attempts to prevail on the Japanese are said to have been rebuffed.

Peter David

US science budget

Congress acts with 1984 in mind

Washington

CONGRESS and the White House appeared last week to be rushing headlong towards confrontation as both the House of Representatives and the Senate approved a 1984 budget plan which will raise domestic spending and reduce defence spending to levels that President Reagan has already described as unacceptable. Under the congressional budget, total spending on federal research and development is expected to fare slightly less well than it would under the White House plan for a 17 per cent increase. But virtually all the reductions would be in defence research, with the civilian share of the science budget showing significant increases.

In the midst of the political confusion surrounding the 1984 budget, a five-year plan to add \$5,000 million to the budgets of six federal agencies which fund university research was unveiled at a Capitol Hill press conference. The initiative, led by two Missouri senators, Democrat Tom Eagleton and Republican Jack Danforth, was devised with help from the Association of American Universities (AAU) and is to be codified in a University Research Capacity Restoration Act.

Senator Danforth conceded that the initiative was unlikely to have a major impact on the 1984 budget process, which is already at an advanced stage. But he said the act would form a blueprint for congressional action over the coming years designed to revitalize American science and to upgrade the quality of university research. He said co-sponsors of the legislation intended to introduce amendments to the spending of six agencies: the National Institutes of Health (NIH), the National

Aeronautics and Space Administration (NASA), the National Science Foundation (NSF) and the Departments of Energy, Agriculture and Defense.

AAU chairman William Danforth (Senator Danforth's brother) claimed that the senator's initiative was the first attempt by a congressional group to look at the needs of the research universities across the whole range of major spending agencies. The initiative is being supported by a number of prominent Democrats, including Senators Edward Kennedy (Massachusetts) and Daniel Moynihan (New York). Moynihan said last week that there is genuine concern about the United States' dwindling lead in science, and complained that the recent breakthroughs at CERN (the European Organization for Nuclear Research) were the first historic events in experimental physics to take place outside the United States since the 1930s.

A major focus of the initiative is an attempt to upgrade the quality of university equipment and laboratories. The group is planning a series of amendments which would provide federal agencies with extra funds to make outright equipment grants to universities and provide additional funds, on a matching basis, to renew or replace existing laboratories. Annual increases for the Department of Agriculture would be \$70 million; for Defense \$135 million; for Energy \$64 million; for NSF \$75 million and for NASA \$40 million. The amount for NIH has not been specified.

Funds would also be provided to increase research grants, to raise the number of graduate fellowship awards and to support young investigators beginning research careers.

Peter David

Acid rain research

Too late for Black Forest?

RESEARCH groups in Munich in the Federal Republic of Germany have formed a consortium to investigate how acid rain is destroying the Black Forest, a great stand of fir and spruce that covers the upper Rhine — and provides one of the major recreational areas of Europe. But the efforts are fifteen years too late, say some: the problem is already so bad that Germany "cannot afford to wait until the scientists have results" one candid member of the new consortium, Professor Peter Schütt, a botanist at the University of Munich, said last week.

According to Schütt the forests of South Germany are deteriorating rapidly. "I don't say they'll be dead in ten years, but by that time there will be effects that will be very noticeable and unpleasant." And the scientific problem is complex, Schütt believes, with no simple solution in view.

Dr Uwe Arndt, now of the University of Ulm, and others warned of possible damage by acid rain 15 years ago, but many German scientists were slow to react. There is no German environmental research council. And the country's major facility for experimentally exposing trees to gases, a regional laboratory near Essen in the Ruhr, has done almost no relevant research, say British observers. Essen's large chambers for the controlled exposure of trees to sulphur dioxide have not been used for four or five years, says one leading German botanist who would have dearly liked to use the equipment.

Research has thus been left largely to others, mainly in the regions of Munich, Karlsruhe and Freiburg, without the necessary large chambers. But this period is over, says the chairman of the new Munich-based consortium, Professor Hubert Ziegler of the Technical University of Munich.

The reason is that the German federal radiation and environmental research association (GSU) based at Neuherberg near Munich has found technical staff (after the closedown of a reactor) and a corner in its budget to support acid rain research. So, says GSU research director Professor H. W. Levi, by spring next year GSU will have working 16 chambers with volumes of 0.8 m³ to 25 m³ ("large enough to step inside and work"), in which it will be possible to control temperature, humidity, gas chemistry, light and draught. "There are similar things elsewhere", said Levi last week, "but we are going to look into the biochemical mechanisms — not just phenomenology." The chamber will be corrosion-resistant, and will be suitable for use with radioactive tracers. The cost will be DM 5.5 million (£1.4 million). "We are hoping for additional support from the Bavarian state government and the federal research ministry [BMFT]", says Levi.

Meanwhile the forest continues to decay, and for complicated reasons. A week ago, for example, Ziegler was surprised to discover that near the Tegensee, a lake a few kilometres south of Munich, dying trees also carried fast-growing lichens. These lichens are normally believed to grow only in the purest air, says Ziegler.

As for symptoms, honey production — a good gauge of the overall health of a forest — has failed catastrophically in the Black Forest, previously the main source of German honey. Old trees — over 100 years old — are the most dramatically affected, losing first their old needles and then their new ones. But young trees in the mixed-age, natural Bavarian forests are thus exposed



Die-back on a Black Forest conifer

to the light, and photo-oxidants may play a key part in the damage, Ziegler believes. Certainly young trees are dying near the tree-line, where they are exposed. Nitrogen oxides in a synergistic mixture with sulphur dioxide are probably the main culprits, together with secondary and tertiary biological effects such as the encouragement of acid-loving fungi, possibly affecting root hairs.

What can be done to counter the threat? Other than research to pin-point causes and suggest therapies (perhaps through key trace element fertilizers), there must be more control of sulphur dioxide and traffic emissions, says Ziegler. "It is most urgent to seek a Europe-wide agreement", he says — understandably, since at least half the pollution is believed to come from foreign sources, such as the French Rhone valley and Switzerland. To reach such an agreement, however, may take longer than the research needed to understand the scientific problem — which would be too late.

Robert Walgate

UK earth science research

SERC threatens new space laser

THE United Kingdom's new satellite geodesy facility — the Satellite Laser Ranger (SLR) at the Royal Greenwich Observatory in Sussex — is threatened with extinction soon after its completion. Its only hope seems to be a rescue by the National Environment Research Council (NERC). The SLR is high on the list of cuts that the Science and Engineering Research Council (SERC) is being forced to make following the recent decline in the value of sterling and the consequent increase in its foreign subscription commitments (see *Nature* 5 May, p.4).

The SLR was developed by Professor S. Ramsden and colleagues in the applied physics and electronics departments of the University of Hull. It can fire 150 ps pulses of green laser light at a rate of ten pulses per second. These are reflected from a satellite (such as the Laser Geodynamic Satellite "Lageos"), received on the ground by a 50 cm reflector and timed from emission to reception with an accuracy of 100 ps. The angular position is measurable to within 25 microradians. For Lageos, orbiting at a height of 6,000 km, the range of the satellite can be determined to within 5 cm.

The system has only recently been installed and tested at the Royal Greenwich Observatory, under the direction of Dr G. A. Wilkins, and the tests have included successful ranging measurements during both night and day (the latter being more difficult to achieve owing to photon contamination from the sky). Initial results have been reported in the bulletin of laser ranging data prepared by the Center for Space Research at the University of Texas, and the precision is of quality comparable with that of other leading facilities such as that situated at the Goddard Space Flight Center, Maryland.

The proponents of the system argue that its data are of value in many disparate fields. Satellite orbits can be used to monitor short-period changes in the Earth's rotation, which can be related to processes in the Earth's atmosphere and in the Earth's interior. They can also be used to provide precise positions of observing stations, allowing continental drift to be monitored. Laser ranging also permits the mapping of the Earth's complex gravitational field, which reflects the distribution of material on and beneath the Earth's surface. Plans for the SLR include participation in two international programmes.

The wide-ranging scope of geodetic data may provide the solution for the SLR's problems. The technical development of the facility has been funded by SERC but much of the present interest in such data stems from solid earth geophysics, which is supported by NERC. The running cost is estimated by SERC to be £85,000 a year,

and the council's Solar System committee has decided that this cannot be afforded given the current financial stringencies.

The final decision, as far as SERC is concerned, rests with its Astronomy, Space and Radio Board. No formal approach has yet been made to NERC about the SLR, although NERC is "aware of the problem" and may reach a formal decision by the autumn. The impression given by an SERC spokesman at a recent Royal Society meeting was that, unless a more coherent geodesy/geophysics programme is put forward in the near future, SERC is reluctantly, ready to wash its hands of its investment. The only other way in which SERC may continue to fund the project is if the consultative committee (involving the Treasury, the Department of Education and Science and SERC) recommends, and the Treasury agrees, that SERC can be reimbursed for the extra money that has had to be spent on foreign subscriptions.

Philip Campbell

Aids for AIDS

Washington

A NEW medical journal devoted to basic research and clinical observation on the acquired immunodeficiency syndrome (AIDS) is to start publication in the United States next month. Its publisher, Mary Ann Liebert, has promised to contribute 20 per cent of the profit from the journal to AIDS research and treatment.

The contribution from *AIDS Research* will take the form of an unrestricted grant to the AIDS Medical Foundation in New York, a non-profit organization set up in May to augment government funding for research on the disease. Dr Joseph Sonnabend, a virologist and microbiologist with a private medical practice in Manhattan, is to be editor of the new journal and chief medical officer of the foundation. Nobel laureate David Baltimore is expected to become a trustee for the foundation.

Dr Mathilde Krim, the foundation's chairman and a member of the Sloan-Kettering Institute for Cancer Research, said the organization intended to raise money from government, private individuals and corporate donors. She said the foundation would keep overheads to a minimum so that most of the contributions received would go directly to research. A spokesman for the foundation said it had received between \$20,000 and \$30,000 towards starting up costs from the Mathilde and Arthur B. Krim Foundation. The Hoffman-La Roche and Schering foundations had also promised to contribute.

The initial priorities of the foundation include the establishment of a repository of biological specimens obtained from volunteer patients and the creation of an AIDS clinic offering free outpatient treatment and counselling. Peter David

UK university research

Industrial links urged

THE British Government should spend at least £15 million a year to encourage universities and other institutes of higher education to forge better links with industry says a new report (Improving Research Links between Higher Education and Industry) commissioned by the Prime Minister, Margaret Thatcher. The report, undertaken by the Advisory Council for Applied Research and Development (ACARD) in collaboration with the Advisory Board for the Research Councils (ABRC), says that the initiative must lie with the universities but that industry must respond.

It suggests two principal ways in which the extra £15 million should be spent. The first it calls a pump-priming fund. The fund of £5 million a year would be used to build up the infrastructure needed for effective cooperation with industry in those institutes of higher education that lack it. The money might, for example, pay for an industrial liaison officer or measures to promote an exchange of staff with industry.

Secondly, there should be an industrial seed corn fund to act as a further incentive to institutes that already collaborate fruitfully with industry and to support their extra collaborative efforts. The seed corn fund, half of which should come from the Department of Industry, would operate on the Matthew principle that to those who already have, more shall be given. Each institute would receive a sum from the fund equal to 25 per cent of that which it earned in the previous year through contracts and consultancies from the private sector and the public trading sector. From figures submitted in evidence the councils estimate that the total sum earned by institutes in that way is currently £40 million a year (an average of £600,000 per university and £80,000 per polytechnic). Within five years, in part through the measures proposed in the report, the councils estimate that the figure will have risen to £100 million.

The report also repeats the oft-heard (from ACARD, the universities and others) call for the British Technology Group to lose its right of first refusal on inventions made at universities with funds from the research councils. The British Technology Group might instead provide professional advice to the institutes on how to exploit their inventions. Other measures to aid exploitation should include the compilation and publication of a comprehensive list of the expertise, services and research programmes available at the institutes and a network of brokers to act on the institutes' behalf.

As to current research in universities and polytechnics, the report says: "We have no desire to undermine excellent research and scholarship of any kind . . . But it must be

admitted that some work may be of less urgency, importance and even interest." So, the report concludes, the institutes need to replace research in "worked-out" areas by areas of greater potential that are also "likely to benefit from the stimulus of applied research allied to them".

In addition, the University Grants Committee should insist that individual universities decide on the proportion of its funds and time it wishes to devote to research (an average of 30 per cent is suggested) so that the committee can allocate an appropriate and identified sum for research to each university and ensure that the sum is used for that.

With the statement that "many academics expressed regret that much of their industrial work was for foreign companies", the report exhorts British industrialists to initiate more links with universities and polytechnics. Its most concrete suggestion in that direction is that bodies on which there is a high level representation of industry should urge firms to establish and maintain such links. It proposes that interested industries might club together in various groups, fostered by the Royal Society for the Encouragement of Arts, Manufactures and Commerce, better, but misleadingly in the context, known as the Royal Society of Arts.

Peter Newmark

A NEWS MAGAZINE FOR INDUSTRY & COMMERCE FROM THE UNIVERSITY OF BIRMINGHAM
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UNIVERSITY & INDUSTRY

THIS MAY NOT HELP YOU AT ALL

But on the other hand it may just give you one or two ideas

CONTENTS

- Professor Edward Marshall and his industry research introduces new computer
- Ways to collaborate with the University of Birmingham & what to ask if you want to know more
- Industry buys a University-designed robot & services from the Centre for Robotics Science
- The mathematics of decision & cost & time factors & Engineering the Birmingham graduates
- Applied basic economics and exporting & A new microprocessor chip
- Courses and seminars & Microcomputer analysis services
- Students and business & Health at Work

U&I—short for University and Industry—is a new magazine published by the University of Birmingham. It will appear three or four times a year, and its aim is to tell managers in industry and commerce something about the ways in which university collaboration might be able to help them. We shall report on case histories of successful collaboration and on services offered by the University which companies have found helpful. We shall also give glimpses of some of the advanced research going on at the University which may point the way for long-range planning. We want to show that there are ways in which the University can help. This is good for the University too, because working with industry and commerce on immediate and tangible problems can only enhance the quality of teaching and research at the University. This first edition of U&I aims to introduce some of the ways we can help. If you have any comments on this issue, or on what you would like to see in future editions, we want to hear from you. Please telephone or write to Frank Affrington, Editor, U&I, University of Birmingham, PO Box 363, Birmingham B15 2TT. Telephone: 021-477 1361 ext. 3566.

BIRMINGHAM University's news magazine *University & Industry* is an attempt to interest a wider section of industry in what is going on at the university. The 8-page magazine is to be distributed three or four times a year with most of the 15,000 copies going to businesses in the East Midlands area. The main difference between *University & Industry* and other information sheets for industry issued in the past is the "popular" style of presentation and the efforts being put into extracting readable stories from the university's departments.

US space programme

Shuttle faces economic reality

Washington

ANY remaining doubts about the virtuosity of the space shuttle were dispelled by last week's flawless performance of the Challenger orbiter. The seventh mission in the shuttle programme was the most ambitious so far and included the successful deployment of two satellites (for Canada and Indonesia) and the first ever retrieval of a space cargo. Back on Earth, however, the shuttle has yet to prove that it can be a commercial as well as a technical success.

The shuttle programme is running two years late and has consumed \$18,000 million in development costs, 31 per cent more than planned. The National Aeronautics and Space Administration (NASA) wants to capture a hefty slice of what it expects to be a lucrative commercial and foreign market for space services. But that means fighting off competition from Europe's Ariane, also basking in the triumph of a successful mission.

NASA's principal worry is whether it can launch enough flights to satisfy the anticipated demand. Repeated requests for money for a fifth orbiter have fallen on stony ground in the Office of Management and Budget (OMB). Meanwhile, estimates of the number of missions possible with a four-orbiter fleet have plummeted. The original plans (based on five orbiters) called for 560 missions in the 12-year life of the shuttle system. The number has dropped to 311 and may slip again.

Vivid proof of NASA's vulnerability came with last week's decision to have Challenger land at Edwards Air Force base in California instead of Cape Canaveral in Florida as planned. A prudent response to poor weather conditions, the decision could add \$16 million and eight days to the shuttle schedule as Challenger is returned to Florida. Both the eighth and ninth flights (including the launch of Spacelab) are expected to be delayed as a result.

NASA's present goal is to reach 24 launches a year by 1988, 30 by 1990 and 40 by 1992. But a recent study by the National Research Council concluded that the chances of reaching 30 launches by 1990 were "impossible or highly improbable" and would be only "marginal" with the addition of a fifth orbiter. The main constraint was likely to be a shortage of spare parts and a high rate of engine failure.

Another disadvantage for NASA in its competition with Ariane is the belief of many potential customers that the Department of Defense (DOD) will exercise its right to jump the queue for shuttle launches, and play havoc with commercial and civilian timetables. NASA does not expect that to happen, arguing that with 114 of the 311 missions already assigned to DOD the Pentagon will have more launch opportunities than it could possibly need.

Foreign customers may take a less

sanguine view of DOD's role, however. Representative Daniel Akaka (Democrat, Hawaii) told a congressional committee last month that Arianespace had already begun to warn potential customers that a booking on the shuttle could be displaced by DOD at any moment. Akaka also cited a 1982 NASA study which reported that the United States could lose up to 94 launches to Ariane by 1994, at a cost of some \$3,000 million in lost revenues.

It is not only DOD that might encroach on the shuttle. Congress and the administration are enthusiastic about building a permanent space station, although they have not yet given NASA approval to go beyond the planning stage. The project could cause a surge in government demand for space on the shuttle, squeezing out foreign and commercial customers.

Aerospace companies which believe there will be too much space business for the shuttle to handle have been trying to persuade Congress somehow to expand launch capacity. One company, SpaceTran (recently bought by Federal Express), offered to pay for a fifth shuttle orbiter in return for rights to sell space on its missions. Others have advocated freeing space on the shuttle by providing DOD with a separate orbiter of its own. A decision has to be taken soon, before the skilled workforce that builds shuttle components is dispersed and the production lines closed.

Meanwhile, the administration has introduced a new variable into the debate by agreeing last month to private sector arguments that US companies should be allowed to take over the United States' own expendable launch vehicles (ELVs). These rockets, including well-tried workhorses

such as Titan and Delta, were to have been phased out with the advent of the shuttle but might now be used to launch commercial satellites into orbit in direct competition with Ariane.

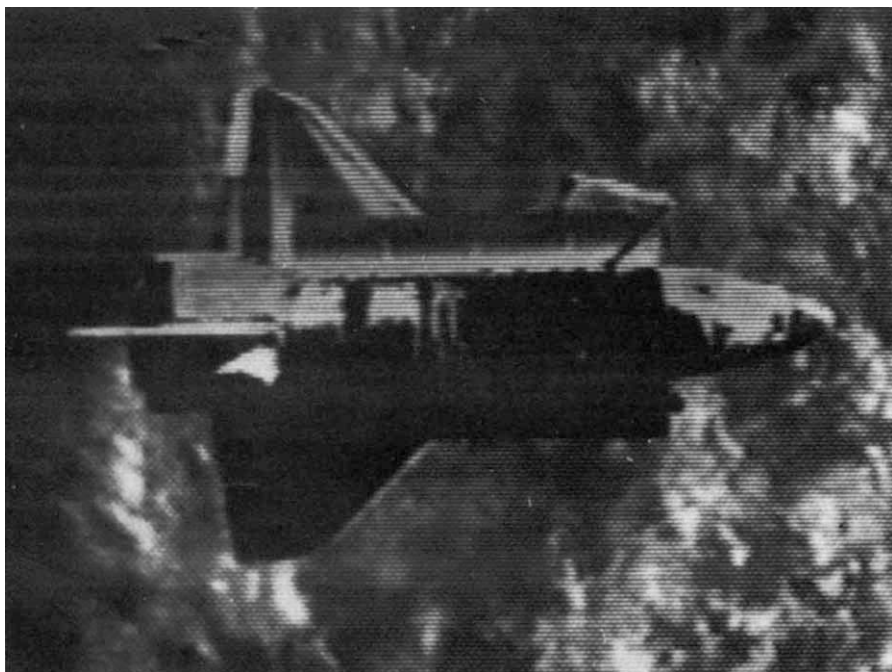
Will they also take business away from the shuttle? That is unlikely, given the vested interest of many aerospace companies in the shuttle's success. Martin Marietta, which will be operating Titan, is believed to earn some \$400 million a year by manufacturing the shuttle's external fuel tanks. But the private sector is already showing signs of wanting to push up the price of shuttle launches so that its ELVs can compete more effectively.

At present, NASA's pricing policy is to recover only "out of pocket" launch expenses. In its latest promotional brochure, NASA claims to be able to launch an Intelsat VI satellite for about \$52 million — less than any other launch system.

The big unknown is whether the hoped-for market for space services will grow as rapidly as expected. NASA and the private sector are optimistic. General Dynamics estimates "conservatively" a need to launch 245 commercial communications satellites between 1986 and 1995, generating about \$10,000 million in launch revenues. SpaceTran puts the commercial and foreign market for space transportation at \$15,000 million between 1983 and 1995, and expects there to be 183 commercial payloads between 1985 and 1995.

Not all experts agree. Members of the Office of Technology Assessment warn that it is always possible that instead of facing more business than they can handle, the shuttle and its competitors will end up with surplus launch capacity. The impact of fibre optics could transform communications economics, in which case the demand for satellite launches could shrivel as quickly as it sprouted.

Peter David



Challenger as seen from the West German Shuttle Pallet Satellite about 200 feet above the shuttle

UK overseas students

Foreign Office steps into breach

THE drastic fall in numbers of overseas students in UK universities which followed the government's decision to charge the "economic" rate for their education now seems to be officially recognized as a worrying trend. In an attempt to improve matters the Foreign and Commonwealth Office is now finalizing plans for how it is to spend some £46 million over the next three years to encourage selected groups of overseas students to come to Britain. The first beneficiaries of the new "focused" policy will start their courses in September.

In 1980 the government decided to charge students from outside the European Community the full economic cost of their courses, and numbers fell by about 25 per cent in two years. "Full cost" fees in clinical medicine, to take the worst case, are now £6,600 per year, while home students pay only £480 per year. Overseas governments made plain their displeasure to the former Foreign Secretary, Mr Francis Pym, and his predecessor Lord

Carrington. Malaysia, which was numerically the country worst affected by the "full cost" policy, retaliated with an anti-British trading policy.

In a remarkable example of cooperation with a non-governmental organization, the Foreign Office encouraged the Overseas Students Trust (OST), an educational charity, to carry out a study on the problem. The trust's 280-page report called for selective assistance for students from British dependent territories (chiefly Hong Kong) and from Cyprus; it estimated the total cost of its proposals at £35 million, of which £15 million would be "new" money.

Responding to the trust's report in February, Mr Pym announced a £46 million package of "targeted schemes" to run for three years. Some £21 million of the total is to come from the unallocated reserve of the aid programme, and £25 million is new money from the diplomatic contingency reserve.

First-degree students from Hong Kong

will, subject to a means test, pay UK home tuition fees from September. The balance of the cost between this and the full cost will be shared equally by the UK and Hong Kong governments, the UK share being limited to £1.9 million in the coming academic year. Up to 1,700 students will benefit from the new scheme, which is now being advertised. Similar arrangements may be made with other dependencies, such as Bermuda and the Cayman Islands: the cost of these schemes would be no more than £100,000 per year.

Cyprus has a "unique combination of claims for special assistance", according to OST, having a large refugee population, no university, and strategic importance to Britain. Mr Pym accepted the trust's case, and the Cyprus government will receive £1 million for each of the next three years to subsidize students coming to the United Kingdom on first degree and equivalent courses. About 700 students will each receive £1,500 in the first year.

The Malaysian government will receive £5 million over three years, £1 million of which is for the coming academic year. This, unlike the Cyprus scheme, will be applied selectively, with the Malaysian authorities doing the selecting. Between 300 and 400 students will benefit next year. The Foreign Office is now confident that all racial groups in Malaysia will be represented among the students coming to this country.

In addition to these special schemes there is to be a new scheme of discretionary awards hopefully aimed at bringing to the United Kingdom future leaders, formers of opinion and able students from potential trading partners. A total of 160 of the new awards will be available this year, at a cost to the government of £700,000; students from all countries will be eligible. Government support for the Commonwealth Scholarship and Fellowship Plan is to be increased by a total of £6 million over three years, and there will be increases for the British Council and the Overseas Development Administration's technical cooperation programme.

Mr Martin Kenyon, OST's director, has welcomed the government's response but says it is only a beginning. Kenyon says the government should maintain and formalize the interdepartmental machinery it established to cope with the overseas students crisis; Foreign Office officials are "actively considering" this suggestion. Ministers are also understood to be favourably inclined to OST's suggestion that universities and other institutions should be allowed to set their own fee levels: fees for many courses would drop if institutions were allowed to charge their own marginal costs with allowances for overheads.

Consultations are taking place with local authorities and the University Grants Committee, but the groundswell of opinion seems to be opposed to such a move.

Tim Beardsley

Soviet space programme

Launch sites are revealed

THE Soviet Union has made a major policy U-turn in its handling of the space programme. For the first time in 26 years, it has permitted two of its launch sites — Kapustin Yar and Plesetsk — to be described in the Soviet press.

Until now, the dateline for all on-the-spot reportage of space launches has been "Baikonur Cosmodrome". The name appears to have been derived from a down-range townlet, although the nearest town to the main launchpad is Tyuratam. Kapustin Yar, although initially the base for the Kosmos series of satellites, has normally only been described as "a launch facility in the middle latitudes of the European part of the Soviet Union".

The Plesetsk base has been even more carefully concealed from the Soviet public. Its existence was first detected, abroad, by the British Kettering group of satellite watchers in October 1966, although the name Plesetsk was first assigned to it a few weeks later by an enterprising American journalist who looked up the coordinates (as announced by Kettering) on a map of the Soviet Union. Until now, Soviet hints of its existence have been more circumspect than in the case of Kapustin Yar — a few references to a "northern launch facility", made within the context of the joint Comecon Interkosmos programme. Here a major security factor may be that this site (south of Archangel) is suitable for launching satellites into polar orbits. When it was constructed in the late 1950s, such orbits were a major cause of international friction, as they enable satellites to scan the whole of the Earth's surface.

Nevertheless, some inkling of these sites did reach the Soviet public. Contrails from Kapustin Yar launches can frequently be seen from tourist sites on the Volga, while Plesetsk launches have been reported by the Soviet public as "flying saucers". Until now, however, such events have been officially explained as "glowing smog" or meteorites.

The decision to identify the sites at last has not led to any major revelations, although *Pravda* has described high altitude tests with the "stratonaut" dogs Al'oin and Kozak — precursors of Laika launched in Sputnik-2 — and some hints of early difficulties in recovering a live payload. The establishment of Baikonur and then Plesetsk apparently resulted in some downgrading of Kapustin Yar, but then, says *Pravda*, a new "glorious page" in its history opened on 14 October 1969, with the launch of Interkosmos-1.

In another article on Plesetsk, *Pravda* concentrates on the flying saucer stories and the difficulties of construction work in so wild a terrain, plus the fact (already well known abroad from the work of the Kettering group) that the majority of Molniya (communications) and Meteor (weather) satellites are launched from Plesetsk, as also were the first French MAS satellites.

Why the Soviets have decided to publicize these launch sites, after so long, is not entirely clear. It may be related to current Soviet attacks on alleged US plans for the "militarization" of space — which would logically demand a greater openness concerning their own space programme.

Vera Rich

Institute for Cancer Research

Slimmed down but healthy

WITH the opening last week of a new £500,000 drug development laboratory at Sutton, in Surrey, the Institute for Cancer Research (ICR) has taken a magic step towards the radical restructuring initiated three years ago by its then-new director, Dr Robin Weiss.

As a result of an 18 per cent cut in funding in 1977, Weiss inherited a £1 million deficit which is being made good by a programme of staff reductions and "retrenchment". A research station at Pollards Wood, in Buckinghamshire, will be closed next year, leaving the institute's activities concentrated at its Sutton site and at the Chester Beatty Research Laboratories in Chelsea, London.

The new laboratory, directed by Professor A.B. Foster, is named after the Cancer Research Campaign, which made capital grants of £900,000 for the building and for improvements to the laboratories at Chelsea. It will house ICR's Drug Development Section, which was formerly split between Sutton and Chelsea, and enable closer collaboration with the Sutton branch of the Royal Marsden Hospital. ICR's Chemical Carcinogenesis Section, headed by Dr Peter Brookes, will move to Sutton from Pollards Wood next year and Dr Julian Peto of the Imperial Cancer Research Fund's epidemiology unit in Oxford will head the ICR epidemiology unit at Sutton from October. The animal breeding centre at Pollards Wood will be run down, and arrangements for the supply of experimental animals have been made with the Medical Research Council.

Weiss's main innovation at ICR has been to expand basic research in cell and molecular biology, which was poorly represented before 1980 and is the subject of important recent developments. With newly-refurbished laboratories, the Cell and Molecular Biology Section is studying human oncogenes and tumour viruses.

Plans are well advanced to supplement those studies with a major new unit devoted to the study of human leukaemia and wholly supported by the Leukaemia Research Fund. Dr Mel Greaves, head of the membrane immunology laboratory at the Imperial Cancer Research Fund, is expected to direct the new unit.

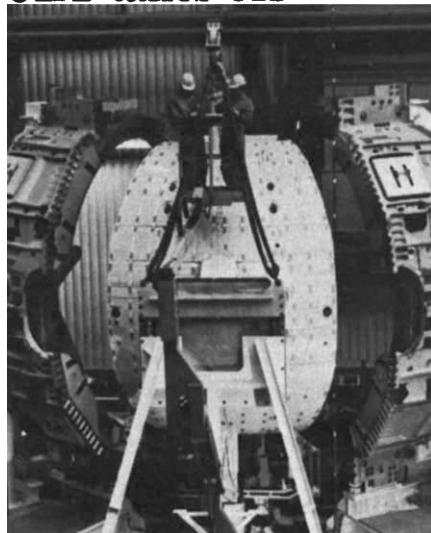
The main casualties of Weiss's restructuring have been tumour immunology, which will continue but on a reduced scale, and radiobiology. Fundamental research in this area has been discontinued, and many of the staff have moved with Professor G.E. Adams to the Medical Research Council's radiobiology unit at Harwell. The teaching role of the institute, as part of the British Postgraduate Medical Federation, has been strengthened with the appointment of Professor G. Westbury to the newly-established chair of surgery. The Cancer Research Campaign has appointed

Dr Tim McElwain to its chair of medical oncology at ICR, and the Medical Research Council has increased its support for this area.

Weiss feels the new slimmed-down ICR, which now has a total staff of 410 compared with 470 three years ago, will maintain its pre-eminent position in those areas where it is best known. New funding arrangements mean ICR now has to compete for research grants from a joint committee of its main sources of support, the Cancer Research Campaign and the Medical Research Council.

Weiss says the new system has provided stability which had been lacking. He points out that the Drug Development Section of the institute has probably introduced more anticancer drugs than any other unit in the world. Two drugs in particular are causing excitement at present: one, known as CB3717, is an enzyme inhibitor which although only in phase I of clinical trials is showing potential for use against cancers of the breast and ovary and could in time become a less toxic alternative to methotrexate. The other, CBDCA, is a non-toxic platinum derivative now in phase II of clinical trials. The section is also continuing its pioneering work on antibody-toxin conjugate therapy, using monoclonal antibodies to target the α subunit of chlorambucil, a widely used antitumour drug, onto *in vitro* bone marrow cells. **Tim Beardsley**

JET takes off



EUROPE'S £175 million attempt to create the conditions for the thermonuclear fusion of deuterium and tritium, the Joint European Torus or JET, circulated its first plasma (of normal hydrogen) on Saturday 25 July. JET, a tokamak, is pictured above towards the end of its five-year construction, which was completed on time and "within a few per cent" of costs envisaged in 1975, the JET team says. □

Lawrence Berkeley Laboratory

Uncertain times for revival

Berkeley, California

ALTHOUGH the Lawrence Berkeley Laboratory's (LBL's) proposal to be the home for a new National Center for Advanced Materials (NCAM) continues to take a beating, it remains the heart of the laboratory's hopes for holding its own in staff and funds. For the latest of a series of studies of the national laboratories, soon to be released by the White House Science Council, is expected once again to call on the laboratories to focus on a more defined mission, more attuned to national needs — a criticism that NCAM is planned to meet.

The recent history of LBL leaves no doubts why NCAM is so important for the laboratory. While many of the other national laboratories were able to cushion the blow of reduced base funding by having single, large projects that could not be abandoned, LBL's diversity left it vulnerable. Over the past two years, LBL has lost 19 per cent of its staff. Ironically, the close association with the University of California's Berkeley campus that has made LBL perhaps the most scientifically solid of the national laboratories (LBL director David Shirley points with pride to the 30 National Academy of Sciences members on his staff) is also responsible for the diversity.

NCAM, when fully operational, would bring the staff and budget of LBL's basic energy sciences division up to a full one-third of the total laboratory. The laboratory employs 4,000 people (2,500 full-time equivalents) with an operating budget of \$110 million. "If we get the support for NCAM," Shirley said in an interview here last week, "we can at best hold the line." The problem is that the plan for NCAM has come under attack. First, materials scientists complained that the proposal was spirited into the President's 1984 budget without sufficient peer review. More recently, the House of Representatives voted to kill all appropriations for the project.

Shirley presses his case for NCAM not only as a salvation for LBL, but also as a model of "serious" efforts to respond to the criticisms of the national laboratories' lack of focus and inattention to national needs. "There is a tendency to lose sight of the necessary coupling between support of research by the federal government and the requirement that research address national needs. NCAM is meant to address these problems — I was not aiming to get a piece of an existing pie, I was aiming to get a bigger pie", he said.

He added that critics of NCAM who claim that it was not peer-reviewed "don't understand the process by which research is supported". He asked a rhetorical question of those critics: "Where were you when we were laying off 19 per cent of our

work force? — that, by the way, was not the result of peer review". And he made no attempt to hide his distaste for "members of the scientific community going after money with all the grace of hogs going after truffles".

The final congressional decision on NCAM is still up in the air. The House of Representatives, after manoeuvring that first took \$5 million away from NCAM for a laboratory at Catholic University (see *Nature* 26 May, p.272), voted to delete all funds for construction from the 1984 appropriations. The Senate has voted to appropriate \$3 million, and the most likely outcome of the House-Senate conference — expected to take place this week — is some compromise between the two figures.

Another large project for which LBL has high hopes is the Tevalac, a proposed relativistic heavy-ion accelerator. LBL's reputation was early staked to its excellence in nuclear physics. But over the years, as the forefront of basic physics has passed to particle, or high-energy, physics, LBL has been left on the sidelines because it has never had one of the large high-energy accelerators.

The Tevalac would recapture for nuclear physics a piece of the action. As conceived in a plan drawn up late last year, it would extend the Bevalac concept — LBL's Bevalac last year became the first machine to accelerate uranium nuclei — to energies of 10 GeV per nucleon at uranium. At these energies, the nuclear physics experiment of colliding nuclei yields a particle-physics payoff: a plasma of quarks and gluons

would be created, which, said Lee Schroeder, one of the Tevalac's developers, could be studied to learn the "thermodynamics" of quarks. This examination of bulk effects of quarks would complement the "microscopic", single quark view that a proposed electron accelerator will offer (see *Nature* 2 June, p.368).

The Tevalac's proponents are hoping for an endorsement by the Nuclear Science Advisory Committee (NSAC) when it meets on July 11–16 to draw up a five-year plan for nuclear physics. NSAC makes recommendations to the Department of Energy and the National Science Foundation on priorities in the field. The Tevalac will be vying with a proposal from Los Alamos National Laboratory to upgrade its LAMF accelerator (the Los Alamos Meson Facility) from 800 MeV to 16 GeV in order to study rare decay modes of certain particles. Brookhaven, CERN and the Gesellschaft für Schwerionenforschung in West Germany are also considering construction of relativistic heavy-ion machines.

LBL planners say the Tevalac would not require additional staff, as savings in personnel would be realized by combining the operation centres of the present Superhilac and Bevalac. The total cost of the Tevalac is put at some \$30 million in research and development expenses and \$100 million in construction. Schroeder said that the most optimistic scenario would have construction begin in fiscal year 1986 and operation in 1990.

Stephen Budiansky

Clinch River fast breeder

The final blow?

Washington

THE roller-coaster fortunes of the Clinch River fast breeder reactor in Tennessee hit a new low last week when the Senate decided to follow the example of the House of Representatives and exclude money for the project from its 1984 energy and water appropriations bill. Jubilant opponents of Clinch River claim that this will finally kill the project unless the nuclear industry offers to pay a much bigger share of the reactor's cost.

Construction of the power plant is expected to cost \$3,500 million, compared with an original estimate of around \$700 million. The industry contribution to the project has remained steady at about \$250 million despite the escalating costs. Congress is expected to renew funding for the programme only if the private sector is willing to raise its contribution to some 40 per cent of the overall costs and shoulder some of the financial risk entailed.

Although the Department of Energy (DOE) was last week considering a new financing proposal from the private sector, officials said the nuclear industry was unlikely to offer much more than the \$800 million proposed in a plan submitted to

Congress in March. And the private sector will probably continue to insist that the government safeguard the industry's investment by promising to repay it through the sale of electricity generated by the plant.

By cutting off money for Clinch River at the end of the year, Congress is flying in the face of the Reagan Administration's policy of continuing work under the existing financial arrangements. The administration had requested \$270 million for work on the reactor in 1984.

Just what will happen at the reactor site if there is no agreement between Congress and the industry remains unclear. Senator Mark Hatfield (Republican, Oregon) said last week that the Senate's intention was to bring all work on the site — including basic preparation of the ground — to a complete halt. But he warned that DOE might be able to continue work if Congress was once again forced to operate under a continuing resolution.

A spokesman for DOE confirmed that the legal status of Clinch River is likely to be ambiguous. Technically, the project remains authorized and there is no specific language in the appropriations bill declaring it suspended. If the project were indeed allowed to die, he added, Congress would have to appropriate some money in 1984 to meet close-down costs.

Peter David

Koestler's last laugh

ARTHUR Koestler and his wife Cynthia, who committed suicide together last March, must have relished the prospect that their bequest to establish the first chair in parapsychology at a British university would set a cat among the academic pigeons. By last week, when news of the £500,000 bequest became known, no university had grasped the nettle, but discussions were in progress with several.

Koestler's last years were marked by an increasing fascination with parapsychology, leading to two books on the subject. He wrote in his suicide note of his "timid hopes of a depersonalized afterlife". Now it is up to Dr John Beloff, senior lecturer in psychology at the University of Edinburgh



and the executor handling the Koestler bequest, to persuade a university to take up the offer. Beloff is negotiating with several universities, including those at Edinburgh and Cambridge, but admits there have been difficulties. The terms of the bequest make it clear that it should fund research into parapsychology itself, rather than, for example, the factors affecting belief in such phenomena. Koestler left no instructions about how the money should be used if early research fails to produce evidence of paranormal forces, and many universities may be reluctant to accept the bequest if it is tied to this field in perpetuity. If it proves impossible to set up a chair, the terms of the bequest allow a number of research fellowships to be established instead.

Mr I Bloomfield of the KIB Foundation, a charity that Koestler helped to set up to fund research beyond scientific orthodoxy, has pledged to put the interest from a sum equivalent to the Koestler bequest towards the research of the proposed professor of parapsychology. Together with the Koestler bequest, this sum would enable a university to set up a unit of perhaps five or six workers. Dr Beloff will also be seeking research funds from the Parapsychology Foundation in New York. No firm decisions have been taken on what facilities would be needed for the new unit, but Beloff is looking for the offer of a laboratory and shared secretarial services, perhaps within an existing psychology department. Disinterested academics may be invited to advise on the provision of equipment. Dr Beloff hopes the chair will be established by October 1984.

Tim Beardsley

Population genetics

SIR — J.A. Shapiro (*Nature* 19 May, p.196) criticizes population genetics on two counts. First, he claims that "there is no evidence" that theoretical population genetics "has been successful in illuminating and explaining the process of biological evolution". Second, he asserts that population geneticists "assume complex (and therefore troublesome) phenomena out of existence"; recently discovered genetic phenomena such as mobile elements are given as an example of this.

The first claim is truly astonishing in view of the many classic works documenting the close agreement between the phenomena of evolution and the expectations of population genetics¹⁻⁶. The second ignores the efforts being made by population geneticists to model aspects of genome evolution, such as the evolution of multigene families^{7,8}, the evolution of chromosomal structure⁹ and the population dynamics of mobile elements¹⁰⁻¹³. We could cite many other papers that show the eagerness of population geneticists to incorporate new discoveries in molecular genetics into their thinking. We hope that molecular biologists will admit the relevance of population thinking to interpretations of the evolutionary significance of their discoveries.

BRIAN CHARLESWORTH

JOHN MAYNARD SMITH

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Advice on AIDS

SIR — I have read with interest your editorials on AIDS and the ensuing correspondence. There is good evidence from animal experiments that contact with semen may promote immune suppression, and frequent anal intercourse may therefore be a causal factor in the immune deficiency characteristic of AIDS.

However, I know of no evidence incriminating promiscuity as such. Would it not therefore be more helpful to suggest that male homosexuals protect their partners by using condoms?

ANNE McLAREN

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Embryological elitism

SIR — I would reverse your editorial writer's argument in the title, "Embryology needs rules, not new laws" (*Nature* 28 April, p.735) to state that the onset of human life needs new laws, not rules. Laws may be transient, but they are longer lasting than rules. Laws are made by people as opposed to rules which are made by an elite, usually for their own interest. The definition of the onset of human identity is not the decision of academic embryologists as your writer claims, but that of our legislators — the politicians, who are influenced by many opinions including religious authority, philosophy and scientists as well as their constituents.

When human life begins in development is a complex argument which is the battlefield of intransigent ideas and emotions. But it does not follow that these ideas and emotions are misguided any more than the concept that human life begins at parturition as if the discarding of the intrauterine environment and placenta somehow gives a freedom to express individuality and therefore personhood. It can be argued that it is only exchanged for the parasitism of parental support and protection which is just reason for denial of human identity according to your editorialist's supposition.

How can academic embryologists be superior to politicians and those who protect human life from conception when they must be influenced by the desire to gain knowledge from manipulation of the human embryo? If the embryo is denied identity or the potential for identity, it can be used in any way. Then there is no need for ethical committees except for consolation of "the general public" so that it can be informed "after the event". Unlike embryology, the making of a definition of human life, in secular terms, belongs to "ordinary people" through the creation of laws.

J.J. MEENAN

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Just imagine . . .

SIR — Ian Fells' derivation of expressions relating lethargy and exergy (*Nature* 14 April, p.566) inspires application of his approach to other fields. For example: progress has two components, (1) materialistic progress and (2) progress in a spiritual sense. As only the latter is associated with increase in *genuine* happiness, we can regard it as the *real* component of progress; materialistic progress on the other hand is the imaginary component.

Likewise there are two ways of arriving at the truth: (1) reasoning from axioms in accordance with previously laid down procedures of inference; and (2) arriving at

the truth by use of man's higher intuitive abilities. The truths deriving from the former approach are inevitably ultimately superseded, and so they constitute the *imaginary* component of truth. The latter procedures on the other hand lead, ideally, to the real component of truth.

Cross-couplings between the real and imaginary aspects of life are weaker than those between real and real and between imaginary and imaginary, so that individuals or groups tend often to exhibit one of these aspects only, to the effective exclusion of the other. *Reciprocal inhibition* plays an important role here. In advanced civilizations there is a dangerous tendency for the imaginary aspects of life to take over and exclude the real (both aspects being necessary for the health of individual societies and of mankind as a whole).

The following is a typical set of processes within the system, that we discover when we examine the situation in detail: science and education play an important role in this cycle. If science and education emphasize the imaginary component of truth, then (it happens in practice) the discoveries which science unfolds tend to be ones which are related to the materialistic (imaginary) component of life, and the progress of society is accordingly largely in the imaginary direction. The loop closes by virtue of the fact that a materialistic society tends to emphasize the *imaginary* aspect of truth in its approaches to education and its approach to life.

An important reciprocal inhibition mechanism is the following: scientists (especially) are prone to condemn strongly (and with considerable emotion) any ideas which are predominantly products of the intuitive mind (notwithstanding the fact that scientists such as Einstein made great use of intuition as a means of seeing their way to the essential form of the truth, in advance of any possible justification of the outcome by rational means). My own experience leads me to infer the existence of a fundamental communication barrier; people trained to think only in terms of rational (imaginary) thinking processes may be quite unable to follow an argument which makes non-trivial use of intuition, such as the ordinary man in the street might be able to follow. Such are the results of educational conditioning. One visible result, for example, is the widespread disregard by the scientific community of the very significant work of Bohm¹.

It is easier to indicate a problem than to suggest a solution. I have no simple one to offer. Perhaps this letter will have a sufficiently disturbing effect on the minds of a few readers that new, freer patterns of thought will emerge in their minds as a consequence; one can but hope this may prove to be the case.

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Names not to be forgotten

It is usually honourable and seldom harmful to attach a name or two to a discovery. But an anarchic system of nomenclature is never helpful.

NOBODY would begrudge Beethoven his fifth, Maxwell his equations or Student his *t*-test. So why should Hardy and Zuckerman not have their virus — the Hardy-Zuckerman 2 feline sarcoma virus described and christened on page 825 of this issue.

Not that everyone would wish to have a tumour virus named after himself or herself, especially one that kills cats. There is, however, a long though erratic tradition of calling tumour viruses after those who first isolated them, beginning with Peyton Rous. The transmissible agent of chicken sarcomas that he described in 1911 came to be known as the Rous sarcoma virus.

The attachment of the discoverer's name to a new beast has, of course, a long history to it, some of it is very respectable and some not so. Many an explorer and collector was by no means unhappy to have his name associated with new specimens he brought home. Some attachments remain, commonly in latinized form (okapi — *Okapia johnstoni*), occasionally as written (Leach's petrel — *Oceanodroma leucorhoa*) and rarely both (Baird's sandpiper — *Calidris bairdii*). Much less glamorous, for the amateur at least, is merely to be the author — the person alone responsible both for the name and the diagnostic description — of a new species (pendulous-flowered helleborine — *Epipactis phyllanthos* G.E. Smith).

These days new mammals are hard to come by and it is a good year when more than one new species of bird is discovered. One alternative is to discover a new comet, customarily named after its original observers. Only a few weeks ago, that convention bore forth Comet IRAS-Araki-Alcock, lamentably giving the Infra Red Astronomical Satellite (IRAS) equal status to George Alcock, an amateur British astronomer, and Genichi Araki, a Japanese schoolteacher. IRAS first picked up what, in retrospect, was the new comet on 25 April. Araki, a Japanese schoolteacher and Alcock, an amateur British astronomer renowned for having memorized in detail much of the night sky, spotted the comet within hours of each other on the night of 3 May.

Another but much less reliable way to posterity for the scientist is through the invention of a technique. The past few years of molecular biology have brought with them, and to a large extent depended upon, the rapid DNA sequencing techniques invented on the one hand by F. Sanger, S. Nicklen and A.R. Coulson and on the other

by A.M. Maxam and W. Gilbert and now universally referred to as the Sanger and Maxam-Gilbert methods. There is also the Southern blotting technique, invented by E. Southern of the University of Edinburgh to identify the presence of defined fragments of DNA within the mixture generated by cutting up a continuous sequence with enzymes. "Blotting" refers to the step whereby an agarose gel, on which the fragments of DNA have been electrophoretically sorted by size, is blotted with a sheet of paper in order to transfer the fragments to a material of appropriate properties for the next step in the procedure. (When the Southern procedure was adapted to suit RNA instead of DNA, some wag coined it Northern blotting, a term that has also stuck.)

Other molecular biologists have their names tagged to fragments of DNA (Okazaki fragments) or particular regulatory sequences of DNA (the Shine-Dalgarno and Pribnow "boxes"). Less obviously the names of H. Bernstein and (Stanford's) S. Cohen are perpetuated — the former, in translation from the German, as the "amber" codon in RNA that terminates protein synthesis, the latter as initials in the legally and biotechnologically important pSC101 plasmid.

Although the vogue now is for terms such as carpet-layer's knee and the sunglass syndrome, pathologists have often left their names with a new disease or syndrome they were the first to describe. It is from that tradition of pathology, which has given us such terms as Kaposi's sarcoma and Burkitt's lymphoma, that the naming of tumour viruses arose. After Rous had published his paper on "A sarcoma of the fowl transmissible by an agent separable from the tumour cells", the sarcomas came to be referred to as Rous sarcomas. Naturally, therefore, when the transmissible agent was identified as a virus, it became the Rous sarcoma virus.

That set a trend but never a habit. Typically what would happen was that a new virus would be described without being named. For example, when J.J. Harvey described how the plasma of a rat with viral leukaemia when injected into mice gives rise to sarcomas, she did no more than call the virus responsible an unidentified sarcoma virus, in part because she was uncertain that it was distinct from a known variant of Rous sarcoma virus. Later, when it became clear that the virus was indeed new, it became known as the Harvey sar-

coma virus (currently of great interest in connection with human oncogenes — see page 775).

The Kirsten sarcoma virus, the Abelson murine leukaemia virus and a host of others acquired their names in the same way. More recently, like W.D. Hardy and E.E. Zuckerman on page 825, some of those who have discovered a new virus have cast aside modesty and donated their names to it rather than wait to see whether others do it for them.

There is no rule that proclaims that tumour viruses should be named after their discoverers. It is not even done widely enough to be called a convention. The acute avian leukaemia viruses never go by their discoverers' names, although there is one, OK10, where OK alludes to N. Okerblom, in whose laboratory it was 10th in a series. The three other members of the group into which OK10 falls are MC29 (29th in a laboratory series of viruses that caused myelocytomatosis), CMII (2nd in a different laboratory's series of viruses that cause myelocytomatosis) and MH2 (the Mill Hill no.2 isolate).

By contrast with the unenlightening results of that system, in which some arbitrary laboratory code determines the virus's name another group of acute avian leukaemia virus, the avian erythroblastosis viruses, tend to be known by the acronym AEV followed by the laboratory code, for example AEV-ES4.

Another alternative is to use the name of the town in which the virus was first isolated. Hence there is the Prague strain Rous sarcoma virus and the Bratislava 77 strain avian sarcoma virus; however, there are also the American discoverer-named Schmidt-Ruppin strain Rous sarcoma virus and the Bryan strain Rous sarcoma virus.

So anarchic a nomenclature "system" is bound to confuse the newcomer or interested outsider. Some kind of rational system of nomenclature is overdue. Perhaps the most sensible is an acronym for the virus type followed by a designation of the isolate by place (and number if necessary). It would certainly be possible to use the discoverer's name instead of the place although, in these days of team work, that would be to encourage internal strife and compound names. Perhaps those who discover a new virus should be honoured in its name but the honour is largely negated when the name is self-promoted.

Peter Newmark

Protein structure

More geese . . .

from Roger Pain

THE story of Mendel's gardener pinching out his peas in order to satisfy his intuitive sense of reality may have its origin in the countryman's legendary closeness to nature, but it is not entirely foreign to the day-to-day activity of the scientist. This week's issue of *Nature* (303, 828; 1983) brings a further essay in the art of observation in the form of a new chapter in the lysozyme story. Most lysozymes, like peas, come in convenient prepackaged containers from which they are easily extracted, and have a good shelf life making them suitable for structural investigation.

To date, lysozymes from a wide range of species have shown reasonable homology and structural similarities, with the exception of phage lysozyme. Despite complete lack of sequence homology, however, the central portion of the molecule supporting the catalytic and binding sites was found to bear a strong conformational homology to the equivalent region in hen egg-white lysozyme — once people had learnt how to look at the structure. This was in keeping with the growing experience that in globular proteins conformation can prove a clearer marker of family relationship than amino acid sequence.

Grütter, Weaver and Matthews, in solving the structure of goose lysozyme (p.828), have challenged the molecular evolutionist with yet more intriguing data. The goose group, which includes the black swan, has longer polypeptide chains than either hen or phage lysozymes and bears no similarity in sequence to either. Again, the central region bearing the active site carboxyls and the substrate-binding cleft is homologous by conformational criteria to the analogous regions in hen and phage. In the sequence to the N-terminal side of this region there is a stretch of 10–20 residues whose α -carbons are positioned in conformational homology with hen, whereas on the C-terminal side, a similar number of residues have their α -carbons positioned in conformational homology with phage. These relationships are summarized in the figure (compare with Grütter *et al.*'s Fig.1).

The authors argue that the conformational similarities in this trio of enzymes support divergence from a common ancestor rather than convergent evolution towards a common function. They also point out, again very reasonably, that the goose enzyme, which has all the features of both hen and phage, could have evolved

from — or into — either; but that hen and phage enzymes could not have evolved directly into one another (see the figure).

The difficulty in this field is that one is faced with competing probabilities in a process which is itself highly improbable, and gene manipulation techniques are not yet sufficiently developed to allow easy multiple experiments that could allow the effects of 'mutations' at potentially interesting loci to be investigated. Problems suggested by, but not exclusive to, these fascinating lysozyme structures include the following. First, the chance coincidence of two sequences of α -carbons, each arranged in aperiodic three-dimensional conformations, is highly improbable. The probability is very much increased however if the α -carbons in each case form a rigid secondary structure such as an α -helix, and more so still if those helices have to depend for stabilization on each being packed against similar conformationally homologous folding units or domains. How does one tell whether the homology in such cases reflects divergent evolution from a common genetic origin, or convergent evolution under selective pressure from conformational constraints?

Second, in describing residues outside the central catalytic region as catalytically 'non-essential', Grütter and colleagues are being careful not to rule out the possibility of functions for these various groups of residues. These functions, which may involve interaction with substrate structures or just stabilization of the catalytic core structure, are at present unknown as also is the selection pressure to retain these groups of residues in their conformation. Fossilization of a region of conformation may occur if further mutation would lead to a different conformation which would decrease the stability of the molecule as a whole, even though its original function is no longer required.

Finally, a familiar problem for the classical morphologist is that, in comparing structures, we readily 'see' similarities and therefore sense continuity. What we cannot see in the molecular fossil record is the possible process of interconversion between dissimilar conformations, sequence clues having already disappeared. These two conformations are therefore regarded as unrelated in a genetic sense. However, it is not difficult to envisage a critical mutation in a stabilizing region of the molecule

which triggers a change in its conformation to one which is still able to interact with and stabilize say the catalytic region.

Given the tools and the insight to make the appropriate mutations, it should be possible at least to visualize some of the possibilities for change in the evolutionary process. In the present state of the art however, Grütter, Weaver and Matthews have taken the useful intuitive course through these problems, as latter-day mendelian gardeners. The only other way leads to frustration and despair:

Farewell all joys, O death come close mine eyes,
More Geese than Swans now live, more fools than wise.

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Oncogenic intelligence

One cancer gene: two mutations

from Peter Newmark

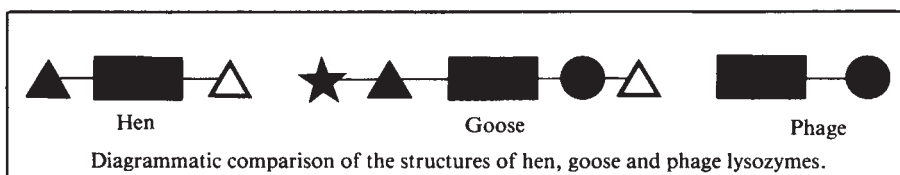
SINCE the discovery, about a year ago, that there was a difference of only one nucleotide (and one amino acid) between the transforming gene of a human bladder cancer cell line and its nontransforming version in normal cells, there has been an unsuccessful hunt for other examples of the same mutation (see, most recently, Feinberg *et al.* *Science* 220, 1175; 1983). Now, instead, a different mutation in the same gene has been found by Yuasa *et al.* in a human lung cancer cell line (see page 775 of this issue of *Nature*).

The gene is that known as c-Ha-ras (or c-bas/has), the cellular gene from which the oncogene of the Harvey murine sarcoma virus is presumed to have been derived. Its product is a 21,000-molecular-weight protein, p21. In the human bladder cancer cell line T24/EJ, a point mutation has changed the twelfth amino acid of p21 from glycine to valine. By contrast the transforming c-Ha-ras gene isolated and cloned by Yuasa *et al.* from the Hs242 human lung carcinoma cell line has glycine at amino acid 12, as normal, but a substitution of leucine for glutamine at amino acid 61.

Since amino acid 61 does not form part of the predicted site whose binding of nucleotides is conceivably affected by a change in amino acid 12 (see *Nature* 301, 262 and 302, 842; 1983), one is, unfortunately, left without even a working hypothesis for why two different mutations should both confer upon p21 the apparent ability to transform NIH3T3 cells.

Are there other point mutations that have the same effect? A series of experimentally engineered mutations throughout the c-Ha-ras gene might provide the answer.

Peter Newmark is Deputy Editor of *Nature*.



Neurobiology

What cloned genes can tell us about nerve growth factor

from Ralph A. Bradshaw

IN the more than three decades since its discovery¹, nerve growth factor (NGF) has proved to be an uncommonly valuable source of insight into the developmental biology of the peripheral nervous system in particular², and growth regulatory molecules in general. It has provided an important pharmacological tool in immunosympathectomy³ through the early administration of anti-NGF antibodies; and it has been instrumental in establishing the importance of other polypeptide growth factors⁴ — indeed, epidermal growth factor was first discovered by Cohen⁵ during his attempts to isolate NGF, and Hendry's discovery of the uptake of NGF by nerve endings⁶ presaged the establishment of receptor-mediated endocytosis of other growth factors and related substances. It has also served to stimulate research on other neurotrophic factors with peripheral and central actions and which may be important in the treatment of neurological disorders⁷. It is therefore paradoxical that the evidence for the actions of NGF, and even its presence, has remained for more than 30 years largely circumstantial. Thus the cloning of the NGF gene by two independent groups^{8,9} has now made possible for the first time a direct approach to these and other questions.

Both groups made use of the known amino acid sequence of the active β subunit¹⁰ of mouse NGF to prepare oligonucleotide primers which identified NGF cDNA in libraries prepared from messenger RNA from mouse submandibular gland — until now the only source of substantial quantities of NGF, for reasons that remain mysterious. The cDNA sequence determined by both groups^{8,9} predicts exactly the same protein sequence, including an aspartic acid assignment for an undoubtedly incorrectly assigned amide in the amino acid sequence of the mature protein¹¹. There is however some doubt about the size of the precursor protein, which contains an extremely long leader sequence that is cleaved from the β -NGF sequence to give the mature peptide. The cDNA sequence predicts three potential initiator methionine positions, occurring at positions -187, -121 and -119, where the first residue of the mature protein is numbered 1. Scott *et al.*⁸ selected the methionine at position -187, predicting a precursor protein of some 33,000 molecular weight, in agreement with a previous study¹², whereas Ullrich *et al.*⁹ have selected position -121 as the actual initiation site. Their argument is largely based on the hydrophobic character of the ensuing residues which is more consistent

with the known structure of other leader sequences.

Meanwhile, Ullrich *et al.*⁹ have gone on to isolate the genomic DNA encoding the human NGF gene, whose structure has also more recently been determined by the Rutter group¹². According to the predicted amino acid sequence, the human protein is quite similar to those of the other higher vertebrates¹³⁻¹⁵. The human genomic data also reveal only one gene copy for human NGF and allow comparison of the location of introns and exons with the putatively homologous genes of the insulin family¹⁶. In fact, the human genomic structure shows an intervening sequence of 6.6 kilobases just upstream from the initiation site suggested at position -121. If the initiation site at -187 is the correct one, then the intron would occur in the 5' precursor segment. As noted by Ullrich *et al.*⁹, one rat insulin gene¹⁷ shows an intron in the corresponding position, though this feature is really not distinctive enough to be taken as strong support for a close relationship with insulin. Further evidence from other gene structures or three-dimensional analyses¹⁸ of the mature protein may be helpful.

A major issue in the processing of the β -NGF precursor is clearly resolved by these studies^{8,9}. It has been suggested¹⁰ that the γ subunit, an arginine-specific protease of the serine family that is normally found associated with β -NGF in the mouse submandibular gland, acts on β precursor molecules to remove a carboxyl-terminal fragment; and indeed Berger and Shooter¹⁹ identified a precursor of the predicted molecular weight if processing were to occur at the double basic sequence found at positions -73 and -72. However, the DNA sequences show that this segment occurs not at the carboxyl but at the amino terminal of the mature protein, with only a dipeptide extending beyond the C-terminal arginine of the mature β -NGF sequence. Although the γ subunit may act at other poly-basic sites in the proposed precursor sequence⁹, it seems equally likely that the subunit is not the major processing enzyme of β precursors. This conclusion is reinforced by the observations²⁰ that guinea pig prostate is totally devoid of γ protein and mRNA despite the fact that it contains high concentrations of mature β -NGF¹⁴.

The cDNA probes of both the mouse and human molecules should also prove invaluable in resolving other major questions regarding the biosynthesis and action of this hormone. Since it was first observed nearly 10 years ago that NGF was specifically taken up at the presynaptic membrane of responsive neurones and

retrogradely transported to the cell body⁵, it has been widely speculated that the physiologically relevant sites of synthesis of the hormone are the cells innervated by these neurones. This idea has received such enthusiastic endorsement that it now represents the basic model, not only for NGF function but also for other neuronotrophic factors, some of which have not even been purified, even though the evidence is largely circumstantial. With the availability of the cDNA probes to β -NGF, it will now be possible to test whether these neuronal end organs are, in fact, producing NGF. The probes can further establish whether NGF is present in the central nervous system. Many observations have suggested possible actions of NGF in the brain but there is little evidence that the hormone is actually found there.

It follows that the cDNA probes should be very useful in elucidating the possible role of NGF in neurological diseases. Changes in NGF concentrations have been found in many pathological conditions²¹ but there is no definitive evidence linking them with the disease. However, observations such as reported by Bell *et al.*²² that deprivation of NGF through transferred immunity can produce a condition similar to that found in familial dysautonomia are highly suggestive. The long-awaited cloning of the β -NGF gene will not only provide a tool for investigating the nature of these neurological diseases, but could clearly also lead to the production of the human β -NGF protein in quantities large enough to be therapeutically useful. □

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Astronomy

Nonradial pulsations of very hot dwarf stars

from Arthur N. Cox

IN this issue of *Nature* (p.781), there is a paper about the pulsations of a very hot dwarf star discovered in 1979. This very blue and very hot star allows us to look into the centre of a highly evolved star that was originally perhaps a few times larger than our Sun. During its evolution over billions of years, the outer layers have been shed by various poorly understood processes, and what now remains is the nuclear processed core laid bare. Of particular interest is the possibility that measurements of slow changes in the observed pulsation periods may be a direct test of our calculations for the cooling of this very hot dwarf star as it becomes a more common white dwarf.

The 14.5-magnitude star, known as PG 1159-035 (the two sets of numbers indicate the approximate right ascension and declination), was discovered in a search for very blue objects that could be quasars. The spectrum of the star reveals that it is in fact a very rare hot star with a surface gravity 10,000 or more times greater than that found on Earth. The spectrum obtained from observations using optical telescopes, the International Ultraviolet Explorer, the Einstein Observatory and the Voyager 1 and 2 spacecrafts shows no peak of intensity for the shortest available wavelength, suggesting a very high temperature, perhaps as high as 150,000K.

No hydrogen can be seen in the spectrum although it would be expected to be present even at these hot temperatures. Helium and carbon seem to be the most abundant elements, at least at the surface.

The most unusual aspect of this star is its luminosity variations which indicate pulsation with periods of about 460 and 540 seconds and with others not yet accurately determined. These periods and their possible changes allow a comparison of observations and stellar evolution theory.

Stellar evolution theory and some observations predict that for stars of mass up to 8 or so solar masses, the central 0.6–1.4 solar mass will be composed of carbon and oxygen at the end of its life. These elements are produced by thermonuclear burning of hydrogen to helium and subsequently to carbon and oxygen. This process involves combination of three helium atoms to form carbon which can be followed by another fusion of a helium atom with the carbon giving oxygen. Calculation of the relative amounts of carbon and oxygen using the latest radiative capture cross-sections gives a small amount of oxygen. But for the star to pulsate, more oxygen, with its cyclical ionization at temperatures above a million kelvin, is needed.

The observed helium signal in the spec-

trum must be from the outer layers which did not completely convert helium to the heavier elements. Although all hydrogen has been completely stripped off from the star, a very small amount of helium remains in the outer 10^{-12} – 10^{-13} of the stellar mass. Helium at greater depth would poison the pulsation driving force and make the star nonvariable.

Following the discovery of this very hot star, some theoretical calculations were done assuming a size of 0.6 solar masses at 5,151 solar luminosities and that the pulsations giving the luminosity variations were radial, that is, purely spherical. The success in predicting the two strongest observed periods and their instability was spoiled when further observations showed periods unaccounted for and broad spectral lines indicative of a very high surface gravity. It became clear that the star had a weaker luminosity (about 100 suns) and a smaller

radius than assumed, not much larger than that of a common white dwarf star. For a star of this small radius — only a few per cent of the solar radius — the radial pulsation periods are very short, typically only 10 seconds. The observed longer periods therefore must be nonradial g-modes of high overtone whose motions are mostly caused by gravity at temperatures near a million kelvin. These details are given in *Astronomical Journal Letters* of 1 May.

An important issue is the amount of oxygen in the pulsation-driving regions buried under only 10^{-10} of the stellar mass. If the star is as hot as the Einstein and Voyager observations indicate, carbon will be completely ionized at this depth. In that case we need to rely on a significant abundance of incompletely ionized oxygen there to drive the pulsations, and that amount is much larger than stellar evolution theory predicts.

It is hoped that the predicted period changes of this star and the few similar ones recently discovered can give further insight into the evolution and perhaps even the composition of very hot dwarf stars. □

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Geophysics

Rare gas isotopes and the evolution of the Earth's mantle

from Grenville Turner

THE structure and major-element chemistry of the Earth's mantle are relatively well understood as a result of the combined evidence from seismology and from studies of the chemistry and petrology of mantle-derived rocks and xenoliths. The dynamics and the consequent evolution through time of the mantle are subject to greater uncertainties, and while geophysicists are united in their acceptance of the importance of convection there is considerable disagreement over the dominant pattern of convection. At their simplest, the arguments centre on whether the convection cells extend throughout the mantle, as a whole or are layered with separate systems for the upper and lower mantle. A paper published in this issue of *Nature* (p.795) now presents important new evidence from gas isotope ratios supporting a layered mantle.

Studies on isotope ratios have played an important part in imposing chemical constraints on allowed models of mantle evolution and convective mixing. The models depend on the existence of suitable pairs of isotopes of a given element, one a 'primordial' isotope present in the Earth since its formation, the other a 'radiogenic' daughter isotope produced by radioactive decay at a known rate throughout geo-

logical time. The isotopic composition of these isotope pairs in different terrestrial reservoirs, for example different regions of the mantle, the Earth's crust and, in the case of the rare gases, the atmosphere, are a function of the transport and mixing of parent and daughter elements between the different reservoirs. These principles have been applied with great success for the elements strontium, neodymium and lead, which are involved, along with the corresponding 'parent' elements rubidium, samarium and uranium, in the differentiation of the mantle to produce continental crust. They have led to a picture of a mantle containing regions (the upper mantle?) that are depleted in crust-forming elements and other regions (the lower mantle?) that are essentially undepleted.

At the same time, rare gas isotope pairs have been investigated in an attempt to place constraints on the evolution of the atmosphere as well as on the corresponding transport process in the mantle. The rare gas radiogenic/primordial isotope pairs are ^4He – ^3He , ^{40}Ar – ^{36}Ar , ^{129}Xe – ^{130}Xe and ^{136}Xe – ^{130}Xe . The radiogenic isotopes are produced respectively from α -decay of uranium and thorium, β -decay of ^{40}K , β -decay of ^{129}I and spontaneous fission of ^{244}Pu and ^{238}U . ^{129}I and ^{244}Pu are referred

to as extinct parents since, having relatively short half lives of 17 Myr and 76 Myr respectively, they are no longer found in nature. They were present however in the primitive Earth as a result of nucleosynthesis immediately before the formation of the Solar System and provide important constraints on the earliest stages of the outgassing of the Earth's atmosphere. Since the outgassing of the atmosphere began at the time the Earth formed, studies of mantle rare gases can, in principle at least, provide information on mantle transport covering the whole of Earth history. The rare gases therefore provide an essential complement to the strontium, neodymium and lead studies which, being related to crust formation, give information for the period during which a permanent continental crust has existed, that is, the last 3,000 Myr or so.

Clear evidence of present-day outgassing of rare gases from the mantle dates back almost a decade with the discovery by Craig and co-workers of excess (primordial) ^3He in the ocean above the East Pacific Rise and in the chilled glassy rims of mid-ocean ridge basalts (MORBs). Since then a large body of helium data has been collected which indicates that the $^3\text{He}/^4\text{He}$ ratio in the source regions of MORBs is about eight times greater than the atmospheric value (the atmospheric ratio is dominated by radiogenic ^4He from the outgassing of continental rocks). In contrast, areas associated with 'plumes' from the lower mantle, such as Hawaii and Yellowstone, have yielded $^3\text{He}/^4\text{He}$ ratios of up to 30 times the atmospheric value, corresponding to a greater proportion of primordial helium and interpreted as representing relatively undegassed, that is more primitive, mantle.

There is also a substantial body of data on the heavier rare gases, in particular argon, though here interpretations are less clear cut because of the much greater difficulty of eliminating the effects of contamination by modern atmospheric gas. By contrast with the heavy rare gases, which are conserved in the atmosphere over geological time, helium escapes into space on a time scale of a million years and atmospheric contamination is not a serious problem. Nonetheless there is clear evidence that the MORB source regions have a very high $^{40}\text{Ar}/^{36}\text{Ar}$ ratio and thus have already lost much of their primordial ^{36}Ar . There is some evidence from Japanese workers that the argon from deep mantle sources is not depleted in ^{36}Ar , though measurements of low $^{40}\text{Ar}/^{36}\text{Ar}$ ratios are always subject to the difficulty of distinguishing a true mantle component from atmospheric contamination.

An unsatisfactory aspect of much of the published data has been that, for technical reasons, different laboratories have specialized either in the helium measurements or in the heavier gases. There are exceptions to this but it is only with the publication of the paper by Allegre and co-workers in this issue of *Nature* that a really

convincing synthesis of helium and heavy rare gas data has been achieved. Moreover these workers have analysed radiogenic-primordial pairs of helium, argon and xenon on samples for which strontium isotope data are also available, making possible a synthesis of mantle isotope studies with respect to both the crust-forming and the atmophile elements.

The samples analysed by the Paris group cover a range of MORB basalt glasses and Hawaiian glasses and show clear correlations between the radiogenic/primordial ratios $^4\text{He}/^3\text{He}$, $^{40}\text{Ar}/^{36}\text{Ar}$, $^{129}\text{Xe}/^{130}\text{Xe}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ indicative of the long-term existence of distinct regions in the mantle with varying degrees of outgassing and depletion. The sample from the Loihi seamount off Hawaii, which shows the most primitive $^4\text{He}/^3\text{He}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ ratios, has $^{40}\text{Ar}/^{36}\text{Ar}$ and $^{129}\text{Xe}/^{130}\text{Xe}$ ratios closest to modern atmosphere which is expected since the atmosphere contains a major part of the Earth's inventory of the heavy rare gases and its composition must in consequence be close to, though not identical to, that of the undegassed bulk

Earth. However observed correlations indicate that this is indeed a genuine observation and not the result of contamination by modern atmosphere.

Potentially the most far-reaching implication of the observed correlations arises from the very short half life of ^{129}I , the precursor of the 'excess' ^{129}Xe in the MORB samples, which implies that the inhomogeneities in $^{129}\text{Xe}/^{130}\text{Xe}$ must have been established in the first 50 Myr of Earth history. Though the authors are necessarily cautious in the interpretation they point out clearly that the material that suffered this major outgassing 4,500 Myr ago seems also to be the source region of depleted MORB. The fact that the depletion of crust-forming elements in the MORB source occurred much later than the xenon loss provides some of the most compelling evidence to date for the existence of a (convectively) layered mantle. Geophysicists take note. □

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Climatology

New light on climate from old isotope ratios

from Pieter M. Grootes

THE regular alternation between a glaciated Earth where ice sheets cover large parts of North America and north-west Europe and conditions similar to those of the present has been documented in fossil records for millions of years. Much of the long-term climatic information has come from study of deep-sea Isotope records from deep-sea sediment cores and ice sheets play an increasing part in the search for proxy-data of climatic change and the study of the 'deuterium excess' d in the isotope records promises to be an especially useful source of new climatic information.

Recent work on deuterium excess has identified additional ties between isotope fractionation and climate, showing, for example, that during the last glacial maximum, around 18,000 yr ago, the relative humidity was about 10 per cent higher than today over oceanic areas, providing moisture for Antarctic precipitation, and that average windspeeds in those areas were about double the current values¹. This sort of approach shows that more climatic information than just temperature can be obtained from an isotope record if the isotope fractionation occurring in the evaporation and condensation of water is considered in detail.

The d term, introduced by Dansgaard², is the constant in a simple empirical relationship between the hydrogen and oxygen isotopic composition of worldwide freshwater sources ($d = \delta\text{D} - 8\delta^{18}\text{O}$ where δ

is the relative difference in isotopic composition between the sample and a standard, expressed in units per ml). Yet neither d nor the numerical coefficient 8 are really constant. Both vary with the conditions of evaporation, vapour transport and precipitation and thus offer insight into climatic processes.

Studies of isotope and mass transport in the turbulent boundary layer above a water surface in the Laboratoire de Glaciologie in Grenoble led Merlivat and Jouzel³ to formulate a series of equations giving d and the $\delta\text{D}/\delta^{18}\text{O}$ coefficient as a function of such parameters as the roughness of the evaporating water surface (governed by wind speed), the relative humidity above the water and the evaporation temperature.

When these equations were applied to the deuterium excess in the 906-m long Dome C Antarctic ice core, d was found to decrease from about 9‰ in the recent interglacial to 4.5‰ in the ice deposited during full glacial conditions. Possible causes include changes in the isotopic composition of the surface ocean water, evaporation and precipitation conditions at the ocean surface, transport from the source area to the site of precipitation and the firnification, that is the transformation from snow to ice. A semiquantitative discussion of the different possibilities indicates changes in the ocean-air boundary layer as the probable cause for the change in d . The

equations then provide an estimated 10 per cent increase in relative humidity over the ocean and a doubling of the wind speed. doubling of the wind speed.

An explanation for the poor or non-existent correlation between precipitation $^{18}\text{O}/^{16}\text{O}$ ratios and seasonal temperature for low-latitude islands comes from Yapp's recent investigation of the influence of the vertical air velocity component and its water vapour mole fraction on the isotopic composition of precipitation⁴. An isotope-precipitation intensity relationship is presented where both the precipitation intensity and the isotopic composition of the rain depend on the temperatures at which condensation begins and ends. The derived equations are applicable if the cooling of the air masses is due entirely to vertical motion, and if vertical velocity, vertical temperature gradient and mole fraction of water vapour are constant over the vertical condensation interval. Isotope ratios of precipitation from two tropical Pacific islands (Johnston and Wake Islands) where the variability in other parameters affecting the isotopic composition is small seem to confirm the predictions. Although factors such as temperature, latitude and altitude generally dominate in determining the isotopic composition of precipitation, Yapp's results identify the influence of other variables, for example the vertical-velocity component of the air and its water vapour mole fraction. These two variables may influence the interpretation of the Pleistocene isotope record, especially since increased humidity and wind speeds were calculated for full glacial conditions by Jouzel *et al.*¹.

This new climatic information on relative humidity and wind speed is important for the understanding of global climatic change, and for tertiary models which link periodicities of $\sim 100,000$, 40,000 and 20,000 yr in temperature measured from deep-sea records⁵ with the eccentricity of the Earth's orbit around the Sun, the tilt of the Earth's axis relative to its orbital plane and the precession of this axis. A quantitative calculation of the effect on climate is needed to prove that the resulting changes in the solar energy input and in the latitudinal distribution of this energy can cause the glacial-interglacial cycles. □

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Animal husbandry

Red velvet and venison

from T. H. Clutton-Brock

LIKE many other exotic species, deer were introduced to New Zealand, which had no indigenous land mammals except for two species of bat, at the end of the nineteenth century¹⁻⁵. Red deer (*Cervus elaphus*) formed the bulk of introductions but fallow, sambar, moose, wapiti, sika, rusa and white-tailed deer were also released. They spread fast and red deer quickly colonized most parts of North and South Islands. By the early 1950s it was clear that they constituted a serious threat to many native plants and animals^{1,6,7}. The understorey of the native beechwood forests was stripped and trampled; the high tussocky grasslands of the Southern Alps were heavily grazed, damaging the habitats of many cursorial birds; and erosion gulleys began to score the steeper slopes.

The situation was saved by a change in control techniques. The widespread use of helicopters allowed hunters to operate in even the most difficult country and to kill far larger numbers of deer than had previously been possible. By the early seventies as many as 100,000 deer were being killed a year and today wild deer numbers have been reduced by over 75 per cent. Meanwhile, New Zealand has developed a major export trade in venison. Recently deer biologists, farmers and exporters from 16 countries met in Dunedin to discuss deer production^{*}.

On established deer farms, 70–80 per cent of breeding hinds wean a calf every year and the value of (live) female yearlings exceeds £400 a head. Thus profits are considerable despite the high costs of the 8-foot fences necessary to enclose the deer.

As meat producers, deer have several advantages over sheep: their meat has a lower fat content, carcass weight represents a higher proportion of live weight (57 per cent versus 45–50 per cent for sheep or 51 per cent for cattle) and haunch, which provides the best cuts, represents a higher proportion of carcass weight (52 per cent versus 40 per cent in sheep)^{8,9}. On some types of pasture, deer are as efficient converters of grass to meat as sheep and have the further advantage of being less susceptible to many forms of parasite infestation. Finally, venison is regarded as a luxury meat by both the main importers (Germany, which imports 20,000 tonnes of game meat a year, and the USA), commanding a higher price than lamb or beef. Farmed venison can meet the most stringent US hygiene requirements and is hard to distinguish from wild venison or even from beef^{10,11}.

But, undoubtedly, the most bizarre aspect of the development of deer farming in New Zealand is the export of what are euphemistically called 'deer products', mainly to Hong Kong and Korea^{8,12-14}. Though deer products include a wide variety of organs, including tails, pizzles, dried blood, heart, fetus and placenta, by far the most important are velvet antlers — cut each year from the living animal before significant ossification has occurred, at around 65 per cent of their ultimate size. Mature red deer stags can produce up to 2.5 kg of velvet antler a year on average, so at £50 per kg, velvet production represents an important component of deer farm profits. Velvet antler is used as an ingredient in Chinese folk medicines.

Most Chinese herbals (pents'ao) take an enthusiastic view of the benefits of eating deer products. One early (200 AD) herbal contends: "Deer velvet tastes sweet, and its property is warm. It is used for treating metrorrhagia, persistent vaginal bloody discharges, lochia, febrile disease, and epilepsy, and also for reinforcing vital energy, strengthening memory and will, generating teeth and delaying the onset of senescence". Two centuries later, the *Pharmacopoeia of the People's Republic* (1977 edition) is only slightly less optimistic and recommends the use of antler or antler products in treatment of lumbago, gonalgia, mastitis, echymosis, carbuncles, tuberculosis, impotence, spermatorrhea, metrorrhagia, frequent urination, wet dreams, vertigo and deficiency of blood. Even the New Zealand scientists are impressed¹², though few have experimented themselves. At the beginning of the Dunedin meeting, all delegates were presented with a 'survival pack' including samples of velvet antler. I'm still waiting for the effects. □

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*A conference on 'The Biology of Deer Production' was held in Dunedin, New Zealand, on 13–18 February under the auspices of the Royal Society of New Zealand and the New Zealand Society of Animal Production.

Gene expression

Protein contacts for promoter location in eukaryotes

from Andrew Travers

WHAT is the mechanism by which RNA polymerase locates a promoter site? In eubacteria, location requires the direct interaction of RNA polymerase with conserved sequences adjacent to the transcription start point. However for eukaryotes the potential for such a strategy of promoter identification must be severely constrained by the size of the genome. In a mammalian nucleus the concentration of a unique DNA-binding site is $\sim 10^2$ times per genome lower than in *Escherichia coli* while the potential number of random DNA-binding sites is 10^3 – 10^4 times greater¹. These factors imply first that in a eukaryote, a DNA-binding protein must have 10^2 – 10^3 -fold higher affinity for its binding site than would be necessary in *E. coli* — assuming all DNA to be equally accessible; and second, that to achieve efficient occupancy the sequence specificity of the interaction relative to random binding must be 10^3 times greater than for *E. coli*. The dilemma posed is made apparent by the i^{X86} mutation of the *E. coli lac* repressor which increases the affinity of the repressor for DNA, at both random and specific sites, by $\sim 10^2$. This repressor binds so tightly to random DNA that its efficiency in locating the operator and thereby repressing *lac* expression is severely impaired².

In eubacteria the ability of RNA polymerase to recognize promoter sequences is conferred by the core polymerase binding to the σ polypeptide independently of DNA binding. Thus in *Bacillus subtilis* replacement of the major vegetative σ factor by analogous polypeptides during endospore formation allows the polymerase to bind to different classes of promoter with different conserved sequences³. For eukaryotes one solution to the dilemma posed by an excess of irrelevant DNA-binding sites would be for a protein that can direct promoter-specific transcription to bind to the promoter site independently of the polymerase. The transcribing enzyme could then itself bind directly to the promoter-recognition protein. This strategy of promoter location could be common to cell types with widely varying amounts of accessible DNA, for example rapidly growing and highly differentiated cells, and would substitute a primary protein-protein recognition for the protein-DNA recognition used by eubacteria. The promoter-recognition proteins in eukaryotes would, like σ factors, be able to interact with both RNA polymerase and with specific sequences in the promoter site^{4,5}.

There is already substantial evidence that the DNA sequences defined *in vivo* as

necessary for the activity and regulation of RNA polymerase II (or B) and RNA polymerase III promoters do not simply direct polymerase binding. Purified eukaryotic nuclear RNA polymerase behaves like the bacterial core polymerase in initiating on any single-stranded region of DNA without apparent sequence preference⁶. Moreover, in the case of polymerase II promoters, Davison *et al.*⁷ have recently shown that the core promoter region containing the TATA box common to most polymerase II promoters binds in the absence of polymerase itself at least one of the several factors necessary for accurate initiation *in vitro*. This binding depends on the integrity of the TATA box and results in the formation of a stable preinitiation complex. Similarly, activity of promoters used by polymerase III for the initiation of 5S RNA, but not of tRNA or other viral polymerase III transcripts, depends on the binding of a protein, TFIIA, to an internal region of the gene determining transcriptional activity and regulation^{8,9}.

Although the experiments of Davison *et al.* suggest that a specific DNA-binding protein is necessary for promoter identification by polymerase II they leave open the question of how the ability to distinguish between different promoters is achieved. The first strong evidence that selectivity can be obtained *in vitro* has recently been provided by Dynan and Tjian¹⁰ who have partially purified a transcription factor that is required for transcription from the simian virus 40 (SV40) early and late promoters but not from other polymerase II promoters tested. It is not yet known whether the activity of this promoter-selecting factor depends on the upstream control sequences specific to the SV40 early promoter but it seems highly probable that the selectivity observed *in vitro* reflects sequence differences between the promoters tested.

How might such a selectivity factor act? One possibility is that a specific DNA-binding protein recognizes the upstream promoter element and interacts by protein-protein contact with either or both the polymerase and the promoter-recognition protein. Efficient binding of the polymerase at the promoter site would require either sequential or simultaneous interaction with both these protein elements. In this way the binding specificity of the promoter-recognition protein could be bootstrapped to provide high transcriptional selectivity using protein-protein contacts for the primary interaction.

A major consequence of invoking protein-protein contact as the primary

mechanism for promoter location by RNA polymerase is that regulation of those interactions could be achieved by altering the protein-protein contact, for example by covalent modification. Such modification would allow, for example, polymerase II to interact with one set of selectivity factors and exclude interaction with a second set. In the case of a gross environmental perturbation, modification might permit binding of polymerase to only one class of gene. Such mechanisms could explain the switching off of transcription of other genes during the heat-shock response in *Drosophila*¹¹ or the constitutive expression of the major human heat-shock gene in the presence of the adenovirus E1a protein¹². There are also hints that modulation of RNA polymerase II interactions may alter the developmental phenotype of particular tissues. For example a class of RNA polymerase II mutants isolated in rat myoblasts fail to undergo terminal differentiation¹³.

This mechanism also has evolutionary implications. For any organism which utilises high-specificity DNA-protein interactions as the primary mode of regulating transcription, substantial increases in DNA content which are not accompanied by compensating increases in the content of specific DNA-binding proteins could be viewed as selectively disadvantageous by decreasing the efficiency of the response to environmental perturbations. For such organisms there would be a selective premium in maintaining a compact efficiently organized genome. By contrast, in eukaryotes, a reliance on protein-protein interactions as the major determinant of transcriptional control would free an organism from the deleterious effects of rapid expansion of the genome and thereby minimize the DNA load caused by the increased amount of competing DNA. Thus the potential for evolutionary flexibility would be increased. Nevertheless, a regulatory mode depending on the modification of multiple protein-protein interactions might respond less quickly to environmental perturbation than one depending on direct alteration of protein-DNA interactions, and consequently would be at a selective disadvantage in niches favoured by many eubacteria. □

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Earth science

The changing shape of the Earth

from W.M. Kaula

THE tracking of Earth satellites continues to produce new findings about Earth properties. The most recent finding, reported in this issue of *Nature* (p.757), uses 5.5 years' laser ranging to Lageos, a 60-centimetre ball of brass covered by retro-reflectors whose retrograde orbit lies at 6,000 km altitude and is inclined at 73° to the ecliptic plane. The analysis demonstrates an acceleration of the node, the point where the ecliptic plane and the orbit of the satellite intersect. The acceleration could arise only from a rate-of-decrease, $\dot{J}_2$, in the oblateness of the Earth of $-3 \times 10^{-11} \text{ yr}^{-1}$. This result was obtained by C.F. Yoder of Jet Propulsion Laboratory and collaborators at JPL and the University of Texas in Austin. An estimate of $\dot{J}_2$ (25 per cent lower) has also been reported by D.P. Rubincam of NASA Goddard Space Flight Center at the Fifth Annual NASA Geodynamics Program Conference and Crustal Dynamics Project Review in January.

This diminution of J_2 requires a motion of mass towards the rotation axis of the Earth, which reduces the difference between the equatorial and polar moments of inertia, to which J_2 is proportionate. The motion most plausibly arises from the Earth's internal adjustments following the last deglaciation, involving resurgence of material beneath the lithosphere towards Laurentia and Fennoscandia, where the great ice caps melted some 100,000 years ago — probably the greatest trauma received by the solid Earth in at least 10,000 years. The decrease in J_2 is consistent with a previously observed increase in the Earth's rotation rate, by conservation of angular momentum. This 'non-tidal acceleration' is itself inferred from satellite observations: first, of the total rotational deceleration, which is the sum of tidal and non-tidal components; and second, of the acceleration of the Moon's orbit, to which the tidal acceleration of rotation is proportionate. The lunar acceleration was a subject of debate for at least a century, and has been determined to two significant figures only recently by Yoder's JPL colleagues J.G. Williams and J.O. Dickey, who analysed 13 years of laser-ranging to the Moon.

While the determination of $\dot{J}_2$ is essentially a confirmation of another datum, it is a significant milestone in that it is the first clear-cut demonstration of a secular change in the Earth's gravity field. Tidal oscillations in the gravity field have, of course, long been measured by gravity meters. But the establishment of long-term trends from surface measurements has been plagued both by instrumental difficulties and by shallow effects of tran-

sitory or local interest, such as ground-water motion.

The determination of $\dot{J}_2$ is the latest achievement of the NASA Geodynamics Program, which is as old as the space age itself — the establishment of the value of J_2 to four significant figures (rather than two) in 1958 was the first precise quantitative result of the space effort. Initially, geodetic inferences from satellites were by-products. But since 1962 there have been a dozen or more spacecraft primarily directed towards determining either precise locations or variations in the gravity field. However, as missions become fewer and farther apart because of the great expense of significant new capabilities, satellite geodesy tends to lose out for several reasons. First, its results are not quickly achieved and appreciated; there are no striking images in the first month, but rather tedious numbers after long and

painstaking tracking and data analysis. Second, the results are often of military interest and hence unavailable. And third, the scientific use is bound up with that obscure and ill-measured entity, the Earth's interior, whose inaccessibility leads to ambiguity of interpretation.

Thus new results in satellite geodesy in the last few years have come not from new missions, but from refinements of tracking systems and data analyses. The leading candidate for a new mission, satellite-to-satellite range-rate (currently named GRM, 'geopotential research mission') was initially proposed in 1969, but at the moment its launch seems unlikely before 1990, even though it will help to elucidate the structure of tectonically important areas such as the Andes and the Alpine belt. Meanwhile, there has been a cessation of more than a year of laser ranging to the Moon from the leading facility, McDonald Observatory in west Texas. □

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NOTES

THE *Union Médicale* of June 2 announces a discovery of the highest scientific interest, and which, if it turns out to be real, will show that prehistoric man is no longer a myth. On piercing a new gallery in a coal-mine at Bully-Grenay (Pas-de-Calais), a cavern was broken into containing six fossil human bodies intact — a man, two women, and three children — as well as the remains of arms and utensils in petrified wood and stone, and numerous fragments of mammals and fish. A second subterranean cave contained eleven bodies of large dimensions, several animals, and a great number of various objects, together with precious stones. The walls were decorated with designs of combats between men and animals of gigantic size. A third and still larger chamber appeared to be empty, but could not be entered in consequence of the carbonic acid it contained, which is being removed by ventilators. The fossil bodies have been brought up to the surface, and five of them will be exhibited at the *mairie* of Lens; the others are to be sent to Lille in order to undergo examination by the *Faculté des Sciences*. Representatives of the *Académie des Sciences* of Paris and of the British Museum having been telegraphed for, are expected to be present.

We have received the Report of the Royal Victoria Coffee Hall, where, as may be known to many of our readers, much good work is being done at the present time in the way of providing cheap amusement every night, free from the temptation to drink and other evils

common to ordinary music halls. Among other experiments being tried are short lectures of the simplest and most popular kind, generally on some scientific subject illustrated by the oxyhydrogen lantern. We are told that a really good lecturer who understands his audience as well as his subject meets with a most encouraging reception, but that very few men of science give their assistance in this good work. We regret this; but we believe it is largely due to the fact that very little is known of the work in question, and that if a general appeal were made to those men of science who occasionally give an account either of their own work or the work of others, many would be found willing to join in the effort which is now being made to interest the working classes in science in what was formerly the Royal Victoria Theatre.

THE National Museum at Washington is one of the best examples in the United States of the practical application of electricity. In so large a building it was found advisable to take advantage of the best means of communication, first being its system of telephones and call-bells, by which those in any room can communicate with every room in the building. Twenty-six telephones are connected by a local telephone exchange, which in turn is connected with the main telephone office of the city. The result is that but three messengers are needed in this vast establishment. The photographic laboratory is independent of the sun, owing to the electric light there used. If one of the 850 windows or 230 doors is opened, a bell rings, and an electric annunciator shows to an attendant at the main office which window or door it is. This system is soon to be applied to every case of specimens. The watchmen at night, also, are kept to their posts by hourly releasing an electric current at certain stations, which pierces a dial and records their visit. The sixteen clock dials are likewise run by electric currents.

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Secular variation of Earth's gravitational harmonic J_2 coefficient from Lageos and nontidal acceleration of Earth rotation

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Analysis of $5\frac{1}{2}$ years of Lageos satellite range data reveal significant residual nodal signatures: an acceleration and annual and semiannual periods. These signatures primarily reflect variations in the zonal gravitational harmonic J_2 coefficient and hence the polar moment of inertia. The implied decrease of $\dot{J}_2 = -3 \times 10^{-11} \text{ yr}^{-1}$ is consistent with both historical observations of the nontidal acceleration of the Earth's rotation and models of viscous rebound of the solid Earth from the decrease in load due to the last deglaciation.

ARTIFICIAL Earth satellites have been used since the dawn of the space age to determine the Earth's permanent gravity field. This field is normally represented as a series of spherical harmonic functions with constant coefficients¹. The zonal harmonics of degree l are designated J_l , and the J_2 coefficient is directly related to the Earth's oblateness. Within the past decade, a special class of satellites (Geos, Starlette, and most recently, Lageos) has been used to determine, in addition to the permanent gravity field, the amplitude and phase of the semidiurnal and diurnal tidal variations in the Earth's nonzonal gravity field^{2,3}. A decade ago Kozai⁴, Paddock⁵ and Wagner⁶ attempted to use artificial satellites to measure either seasonal (Kozai) or secular (Paddock, Wagner) changes in the gravity field. However, the tracking accuracy necessary to detect these small variations was not then available. More recently, Bender and Goad⁷ and Gaposchkin⁸ have discussed or used Lageos' data to detect the seasonal and tidal (≤ 1 yr) variations caused by similar signatures in J_2 .

We show here that Lageos' orbit (particularly its node, Ω) is sensitive to secular (and periodic) changes in J_2 , especially $\dot{J}_2$. The $\dot{J}_2$ derived from the observed secular acceleration $\dot{\Omega}$ is consistent with the nontidal acceleration of the Earth's rotation⁹. The $\dot{J}_2$ expected from viscous rebound following the last great deglaciation^{10,11} are examined, and this mechanism is shown to be a plausible explanation for the observed effects.

The passive satellites Lageos¹² and Starlette are particularly well suited for geodynamical studies as both are accurately ranged from the Earth using pulsed laser beams. Also, the design of these satellites was chosen to minimize the effects of air drag and light pressure. Their orbits lie well above the dense atmosphere and both have small cross-sectional area to mass ratio A/M . This ratio and the orbital parameters (semimajor axis a , eccentricity e , inclination I , orbital period P , periods of circulation of node $P(\Omega)$, and argument of pericentre (w), $P(w)$) are given in Table 1. Figure 1 describes the orientation of Lageos' orbit with respect to the Earth.

Lageos' node and Earth rotation

It has been assumed that the Lageos orbit would provide a stable benchmark for the determination of the nonlinear changes in the accumulated rotation angle of the Earth (effectively UT1) by measuring the orientation of the Earth with respect to Lageos' node Ω . This section demonstrates that both UT1 and Ω show proportional, similar sized responses to J_2 variations, so this view must be revised if these variations can be detected. When compared with a separate source of

UT1 the Lageos tracking data provides the opportunity to detect J_2 variations and discriminate sources of UT1 variation which arise from J_2 changes from those which do not. Tidal deformation¹³⁻¹⁵, seasonal changes in groundwater¹⁶ and mean sea level and viscous rebound of northern Canada^{17,18} after the last deglaciation are mechanisms which produce changes in UT1 in response to changes in J_2 . A change in the Earth's volume in response to a change in the gravitational constant, thermal expansion of the oceans¹⁹, exchange of angular momentum between atmosphere and mantle²⁰ or fluid core and mantle²¹, and exchange of angular momentum via tidal torques with Sun and Moon are mechanisms for UT1 change which have small effect on J_2 . Of these mechanisms only angular momentum exchange with the atmosphere and tidal deformations have been previously detected.

A satellite's node, argument of perigee w and mean anomaly l are primarily sensitive to variations in the even zonal harmonic

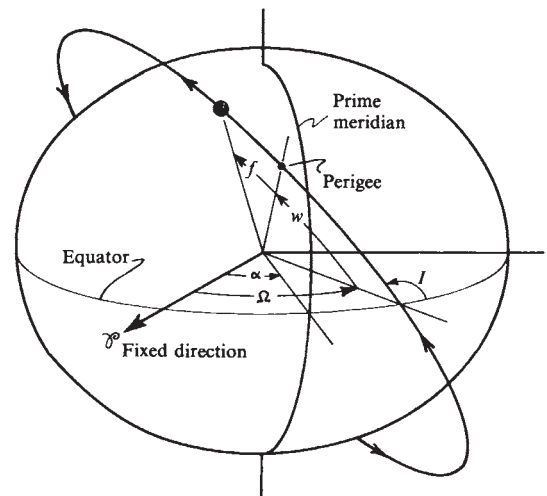


Fig. 1 Geometrical relationships and definitions: the angle α , which is nearly a linear function of UT1, measures the Earth's rotation with respect to a fixed direction, $\mathcal{F}$. The longitude of the ascending node, Ω , and the inclination, I , determine the orientation of the satellite's orbit plane. The argument of perigee, w , is measured in the orbit plane from the equator to the orbit's point of closest approach to the Earth.

coefficients. The node may also be affected by unmodelled variations in inclination which would alter our estimates of the variation in J_2 . The following dynamical equation¹

$$\frac{d}{dt} \delta \Omega = -\frac{3}{2} n \left(\frac{R_e}{a} \right)^2 \frac{\cos I}{(1-e^2)^2} (\delta J_2 + \delta J_4 f_4 + \delta J_6 f_6 - \delta I \tan I J_2) \quad (1)$$

$$f_4 = \frac{5}{8} \left(\frac{R_e}{a} \right)^2 (7 \sin^2 I - 4) \frac{(1 + \frac{3}{2} e^2)}{(1-e^2)^2} \quad (2)$$

$$f_6 = \frac{35}{64} \left(\frac{R_e}{a} \right)^4 (8 - 36 \sin^2 I + 33 \sin^4 I) \frac{(1 + 5e^2 + \frac{15}{8} e^4)}{(1-e^2)^4} \quad (3)$$

describes the perturbation in Ω caused by the variations δJ_2 , δJ_4 and δJ_6 and δI . Here $n = 2\pi/P$ is the orbital mean motion and $R_e = 6,378$ km is the Earth's equatorial radius. For Lageos, the inclination factors $f_4 = 0.37$ and $f_6 = 0.08$ are sufficiently large that we must be concerned with the magnitude of δJ_4 and δJ_6 relative to δJ_2 predicted by various mechanisms. We shall argue later that the assumption that δJ_2 dominates in equation (1) is a reasonable first approximation.

The variations in w , l and Ω caused by δJ_2 are proportional. The approximate relations for small e are

$$\delta w(J_2) = -\frac{(4 - 5 \sin^2 I)}{2 \cos I} \delta \Omega(J_2) \quad (4)$$

$$\delta l(J_2) = -\frac{(2 + 3 \sin^2 I)}{2 \cos I} \delta \Omega(J_2) \quad (5)$$

In principle, all three parameters are sensitive to δJ_2 , but the mean anomaly always suffers sizeable irregular variations caused by mechanisms such as variable air drag. For an elliptical orbit the perigee motion would be usable, but Lageos' orbit is nearly circular and the range measurement is sensitive to the product $e\delta w$. Thus, the node is the practical parameter to investigate for Lageos.

δJ_2 is directly proportional to the variation in the polar moment of inertia C for deformation mechanisms which preserve volume²² such as tidal deformation and mass redistribution over the Earth's surface. The relationship between δJ_2 and δC for these mechanisms is

$$\delta J_2 = \frac{3\delta C}{2MR^2} \quad (6)$$

where R is the Earth's mean radius. Variations in Earth spin rate ω directly follow from the conserved angular momentum, $C\omega$.

$$\frac{\delta \omega(J_2)}{\omega} = -\delta J_2 \frac{2MR^2}{3C} \quad (7)$$

Denoting the variations in UT1 driven by δJ_2 as $\delta \text{UT1}(J_2)$

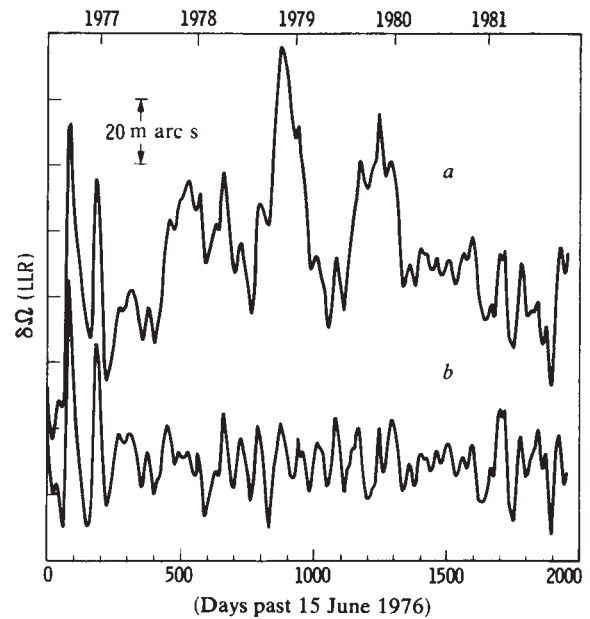


Fig. 2 Residual $\delta \Omega(\text{LLR})$ after removal of the five-frequency (a) and eight-frequency solution (b).

[that is, integrated $\delta \omega(J_2)$], we find

$$\delta \Omega(J_2) = K \delta \text{UT1}(J_2) \quad (8)$$

$$K = \frac{9}{4} \frac{C}{MR^2} \frac{n}{\omega} \left(\frac{R_e}{a} \right)^2 \cos I \quad (9)$$

The above formula applies only for a solid Earth, including oceans. A rotationally-decoupled mantle and fluid core slightly modify the constant of proportionality K (ref. 15). For Lageos, the appropriate K for equilibrium tidal deformations varies from -0.477 to -0.437 for a rotationally decoupled and coupled fluid core, respectively. The latter value is appropriate for slow secular changes in J_2 . Lageos' node moves about 7 m arc s in response to a change in $\delta \text{UT1}(J_2)$ of 1 ms of time (1 ms = 15 m arc s).

Data analysis

Fits of the Lageos tracking data to sophisticated dynamical models still leave residual signatures in the orbital parameters. Since signatures in the node and errors in the adopted UT1 are indistinguishable, two independent sources of UT1 were used in this analysis. Five nodal periodicities were associated with diurnal and semidiurnal tides on the Earth and correspond to small ocean-tide corrections to the nominal tidal model used.

Table 1 Orbital parameters

Satellite	Period (h)	a (km)	e	I (deg)	$P(\Omega)$ (days)	$P(w)$ (days)	A/M ($\text{cm}^2 \text{g}^{-1}$)
Lageos	3.758	12,270	0.004	109.94	1046	-1707	0.0069
Starlette	1.735	7,330	0.020	49.8	-109	93	0.0096

Table 2 18.6 yr, annual and semiannual amplitudes (in m arc s): solution for Lageos' node compared with nominal tidal model and seasonal models

	$\sin \Omega_t$	$\cos \Omega_t$	$\sin \odot$	$\cos \odot$	$\sin 2\odot$	$\cos 2\odot$
$\delta \Omega(\text{LLR})$	-96 ± 88	-151 ± 22	-13.8 ± 1.5	12.2 ± 2.0	-4.4 ± 1.5	-2.6 ± 1.5
$\delta \Omega(\text{BIH})$	-68 ± 109	-177 ± 29	-11.5 ± 2.0	-3.1 ± 2.7	-3.7 ± 2.0	-1.1 ± 2.0
Nominal tidal model						
Ref. 15	1,160	0	11.0	-0.4	-32.4	-12.2
Nontidal seasonal model (δJ_2 only)						
Ref. 14			-1	24	-0.7	-2.5
Ref. 13			-17	21	0	-1.7

Solar angle $\odot = 0^\circ$ on 1 January.

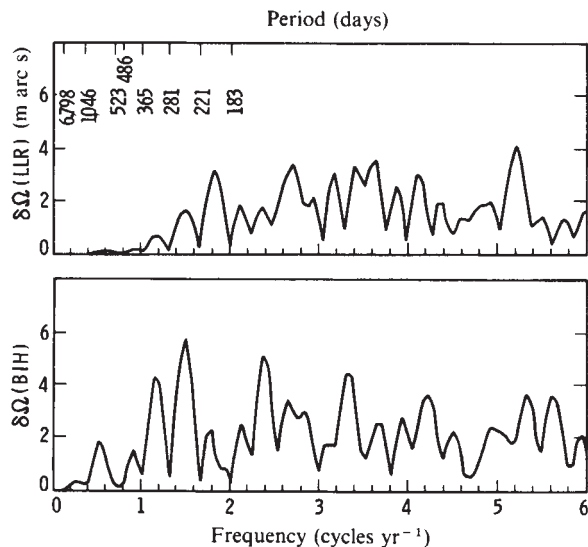


Fig. 3 Amplitude spectra for $\delta\Omega(\text{LLR})$ and $\delta\Omega(\text{BIH})$ after removal of the eight-frequency solution.

In addition annual and semiannual periodicities and a large acceleration were present in the node and these constitute the evidence for the J_2 variations that we are seeking.

Lageos laser ranges were analysed between May 1976 and December 1981. The model included forces from the irregular gravity field of the Earth, solar, lunar and planetary (Venus through Saturn) gravitational perturbations, drag, solar radiation pressure, and nominal tidal expressions³. The nominal amplitudes for 18.6, 1 and 1/2 yr tidal signatures, which have been removed from Ω , are given in Table 2. UT1 was modelled with Bureau International de l'Heure (BIH) circular D, 5-day smoothed values, but polar motion was solved for at the same intervals. After this $5\frac{1}{2}$ yr fit, the corrections to orbital elements were derived at 5–10 day intervals from the range residuals to display perturbations unrepresented in the model. Corrections to the 'node' really reflect corrections to $\Omega - \text{UT1}$, while the errors in both the Ω measurement by Lageos and independent measurements of UT1 by other techniques are comparable in size. UT1 derived by the techniques of lunar laser ranging (LLR) and very long baseline interferometry (VLBI) have demonstrated submillisecond (of time) accuracies^{23,24} and are expected to possess good long-term stability. Consequently a second set of node corrections was derived from the UT1 differences between one of these (LLR) and BIH. The smoothed node corrections are designated $\delta\Omega(\text{BIH})$ and $\delta\Omega(\text{LLR})$. The corrections will be used to infer the equivalent changes in the gravity field using the theoretical $\delta\Omega$ in equation (1).

Both the node corrections using BIH UT1 and the irregularly spaced UT1(LLR)–UT1(BIH) values had nonuniform uncertainties. These quantities, $y(t_i)$ with uncertainties σ_i , were smoothed and interpolated at 5-day intervals of time t using weighted gaussian smoothing.

$$Y(t) = s(t)^2 \sum_i [y(t_i)/\sigma_i^2] \exp[-(t-t_i)^2/2\tau^2] \quad (10)$$

$$s(t)^{-2} = \sum_i (1/\sigma_i^2) \exp[-(t-t_i)^2/2\tau^2] \quad (11)$$

where $s(t)$ is an uncertainty associated with the smoothed quantity $Y(t)$. The sums were limited to ± 35 days around t with a choice of $\tau = 10$ days (FWHM = 23.5 days). This filters out more than half of the amplitude for spectral periods shorter than ≈ 60 days while for UT1 (BIH) the half amplitude point is reached at ≈ 100 days. The smoothed tabulations of UT1(LLR)–UT1(BIH) were subtracted from $\delta\Omega(\text{BIH})$ to get $\delta\Omega(\text{LLR})$, the error in the latter being taken from the quadratic sum of the other two errors.

Spectra of $\delta\Omega(\text{BIH})$ and $\delta\Omega(\text{LLR})$ showed that tidal corrections to the nominal ocean model were necessary. Consequently we solved for empirical corrections (two components each) due to the (nonzonal) diurnal and semidiurnal ocean tides which cause orbital perturbations at periods of 1045.6, 522.9, 485.5, 280.7, and 221.3 days. A straight line was included to absorb a constant J_2 uncertainty. The residuals from the five-frequency (12-parameter) weighted least squares fit to $\delta\Omega(\text{LLR})$ are shown as Fig. 2a. In addition to the obvious curvature, there is a sizeable annual and a detectable semiannual term which we interpret as originating from J_2 variations. Eight-frequency (18-parameter) solutions included 1/2-, 1-, and 18.61-yr periods. The amplitudes are given in Table 2 with the nominal zonal tides which were originally removed. The post-fit residuals for $\delta\Omega(\text{LLR})$ are displayed in Fig. 2b. The curvature and seasonal signatures have been removed. The two peaks near the beginning of the data span have low weight. They match a stretch of sparse Lageos' data and a gap in the LLR measurements. The weighted post-fit r.m.s. residuals for $\delta\Omega$ are 10 and 7.7 m arc s using the BIH and LLR UT1 respectively. We had to accept the restriction that the $5\frac{1}{2}$ yr data span does not permit separating quadratic and 18.6 yr (Ω_4) terms, though physical causes exist for both. At the middle of the data span the 18.6-yr terms yield accelerations of

$$\delta\ddot{\Omega}(\text{LLR}) = -14.7 \pm 1.3 \text{ m arc s yr}^{-2} \quad (12)$$

and

$$\delta\ddot{\Omega}(\text{BIH}) = -18.2 \pm 1.6 \text{ m arc s yr}^{-2} \quad (13)$$

The amplitude spectra of the residuals from the eight-frequency fits are shown in Fig. 3. It is evident that the low-frequency components of the residuals are reduced when the LLR values of UT1 are used. This is in accord with the spectra of UT1(LLR)–UT1(BIH), which decrease from low to high frequency as shown by Fliegel *et al.*²³. The high-frequency end of the spectra also appears to be improved using UT1(LLR). This suggests that the Lageos and LLR data are detecting real variations in UT1 at 60–90 day periods. These frequencies have largely been filtered out of UT1(BIH). We do not know the cause of the peak at 70.2 day, but note that it is 1/8 the circulation period of Lageos' node relative to the Sun. In generating the $1-\sigma$ uncertainties of Table 2, we have taken a noise level of 2.0 and 1.5 m arc s from the spectra of $\delta\Omega(\text{BIH})$ and $\delta\Omega(\text{LLR})$, respectively. The post-fit, r.m.s. residuals can be adjusted for the noise absorbed during the fits to conclude that the real weighted r.m.s. differences are respectively 12 and 9 m arc s when BIH and LLR values of UT1 are used.

The inclination residuals have also been fit with the five nonzonal tidal frequencies. The earliest data show a negative bias in the residuals. If a linear trend is included in the fit then $\delta\dot{I} = 1.9 \pm 0.7 \text{ m arc s yr}^{-1}$, equivalent to a positive $\dot{\Omega}$ of $11.5 \text{ m arc s yr}^{-2}$. We suspect incomplete separation of inclination and polar motion with the earlier, less accurate data. If we remove the first year of data, $\delta\dot{I}$ is reduced to $0.1 \pm 0.3 \text{ m arc s yr}^{-1}$ and is insignificant. However, the shorter data set results in only modest changes in $\delta\ddot{\Omega}$ (for example, $\delta\ddot{\Omega}(\text{LLR}) = -14.3 \pm 2.4 \text{ m arc s yr}^{-2}$).

Rubincam³⁷ reports a substantially smaller value for the secular acceleration ($\delta\ddot{\Omega} = -10.8 \pm 2.3 \text{ m arc s yr}^{-2}$) based on an independent analysis of Lageos' ranges. The source of the apparent conflict is not yet understood.

Tidal dissipation and $\dot{\Omega}$

The lunar node Ω_4 passes through 168.7° at the midpoint of the data span. The very significant $\cos \Omega_4$ correction term mimics a term proportional to the square of the time t . Physical mechanisms for both signatures exist.

By far the largest zonal tide variation in Lageos' node (see nominal values in Table 2) or UT1 is the 18.6-yr tide associated with the precession of the lunar node, but most of this tide was originally removed with the nominal model. A $\cos \Omega_4$ term would arise from a phase shift of the nominal, tidally driven

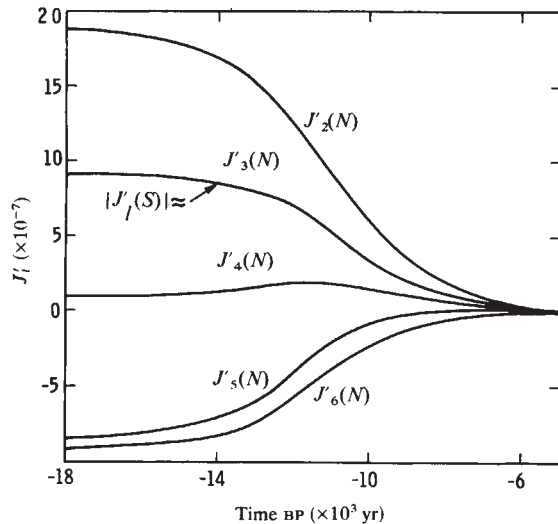


Fig. 4 Elastically compensated load coefficients $J'_l(N)$ and $J'_l(S)$ associated with melting history of Laurentide–Fenoscandia and Antarctic ice sheets, respectively.

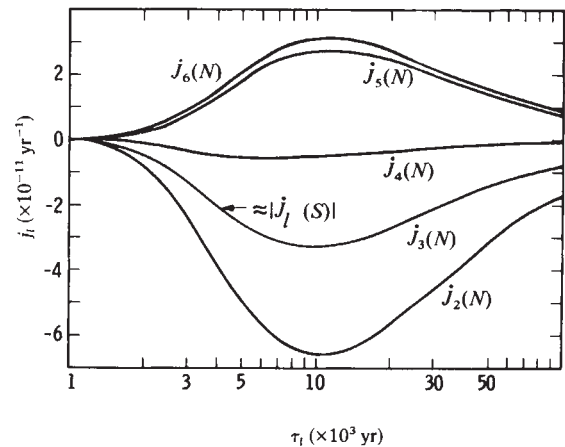


Fig. 5 Present $J'_l(N)$ and $J'_l(S)$ as function of τ_l based on melting history and equation (18).

$\sin \Omega_t$ term and this phase shift depends on dissipation by solid body friction or ocean currents. Dissipation causes a time delay T in the tidal variation of δJ_2 . If T were about 0.4 yr or the dissipation factor $Q \approx |1/\Omega_t T| \approx 8$, then tidal dissipation could account for the entire signature. The ocean tides contribute 12% of the amplitude of the nominal zonal tide model, but they are both the major uncertainty in tidal calculations and the expected source of any significant dissipation since the mantle Q is probably >100 . Even if the oceanic Q were near unity, it would only account for about half of the observed $\cos \Omega_t$ signature. Such large oceanic dissipation is usually deemed unlikely based on Proudman's²⁵ estimate of dissipation by bottom friction. Physical measurements of the amplitude and phase of the 18.6-yr ocean tide height are highly uncertain^{26–28}, but indicate large phase shifts from equilibrium at different tide gauge stations. However, Rossiter²⁷ finds that the mean phase is near zero. He also finds that the mean amplitude of the 18.6-yr ocean tide is depressed by a factor of 2/3 while Currie²⁸ finds that the amplitude is near its equilibrium value. By elimination we conclude that most of the curvature seen in Lageos' node is a secular acceleration with only a small, unknown part related to dissipation in the 18.6-yr tidal constituent. The driving J_2 variation is thus dominantly a rate of change of nontidal origin.

Deglaciation and $\dot{J}_l$

Earth has experienced several ice ages which recur on a 10^5 -yr time scale. 20,000 yr ago, massive ice sheets up to 3 km in thickness covered much of northern Canada and Scandinavia with a total mass $\approx 3 \times 10^{22}$ g equivalent to an 80-m change in sea level. At the same time, the Antarctic ice sheet was considerably thicker than at present^{32,33}. Several investigators^{10,11} have suggested that the Earth's gravity field still possesses memory of the last melting episode which ended about 5,000 yr ago. Also, deglaciation may be responsible for the ≈ 2 m arc s yr^{-1} secular polar motion as well as the nontidal acceleration of ω (refs 11, 17, 18). This section demonstrates that the rate of change of J_2 is compatible with the glacial rebound hypothesis and predicts a nontidal acceleration of Earth rotation in reasonable accord with historical values.

With the observed acceleration in equations (12) and (13) for the two UT1 sources, we derive from equation (1) secular changes in J_2 of

$$\dot{J}_2(\text{LLR}) = (-3.5 \pm 0.3) \times 10^{-11} \text{ yr}^{-1} \quad (14)$$

$$\dot{J}_2(\text{BIH}) = (-4.4 \pm 0.4) \times 10^{-11} \text{ yr}^{-1} \quad (15)$$

Equations (1), (14), and (15) predict the following nontidal

acceleration in Earth rotation, $(\dot{\omega}/\omega)_{\text{NT}}$.

$$(\dot{\omega}/\omega)_{\text{NT}}(\text{LLR}) = 7.1 \pm 0.6 \times 10^{-11} \text{ yr}^{-1} \quad (16)$$

$$(\dot{\omega}/\omega)_{\text{NT}}(\text{BIH}) = 8.8 \pm 0.8 \times 10^{-11} \text{ yr}^{-1} \quad (17)$$

Both are in agreement with Morrison and Stephenson's⁹ determination $(\dot{\omega}/\omega)_{\text{NT}} = 8.4 \pm 1.3 \times 10^{-11} \text{ yr}^{-1}$ using Babylonian lunar eclipse timings. Their estimate is sensitive to the adopted values for the lunar tidal acceleration $\dot{n}_l$ (ref. 29) and the contribution of the solar tide to the tidal $\dot{\omega}$. Their value for the Earth's nontidal acceleration (really a ≈ 25 -century average) is reduced to $(\dot{\omega}/\omega)_{\text{NT}} = 6.3 \pm 0.9 \times 10^{-11} \text{ yr}^{-1}$ using improved estimates for $\dot{n}_l$ from LLR³⁰ ($\dot{n}_l = -2.52 \pm 0.12 \text{ m arc s yr}^{-2}$) and the Earth's tidal acceleration: $(\dot{\omega}/\omega)_{\text{T}} = -26.4 \pm 1.4 \times 10^{-11} \text{ yr}^{-1}$. We can also improve these estimates of $(\dot{\omega}/\omega)_{\text{NT}}$ in equations (16) and (17) by correcting in equation (1) for the secular $\dot{J}_4$ and $\dot{J}_6$ changes predicted by the rebound hypothesis.

The viscous response of the solid Earth to this changing surface load is approximately described by the following relaxation equation

$$\left(\frac{d}{dt} + \frac{1}{\tau_l}\right) J_l = -\frac{d}{dt} (J'_l(N) + J'_l(S)) \quad (18)$$

where $J'_l(N)$ and $J'_l(S)$ refer to the elastically compensated load coefficients associated with the Fenoscandia–Laurentide and Antarctic ice sheets, respectively. The complexities of rigid lithosphere and fluid core are serious complications which are missing from this simple equation, but these effects are not expected to change the final result by $>30\%$.

The time history of the $J'_l(N)$ coefficients shown in Fig. 4 were derived from Wu and Peltier's³¹ Ice-2 maps which describe the recession of the northern ice caps from 18,000 to 4,000 yr BP. The time history of the Antarctic sheet is less certain³². From Hughes *et al.*^{32,33} map of the excess ice covering Antarctica 18,000 yr ago, we find that the initial $J'_l(S) \approx (-1)^l 9 \times 10^{-7}$. If the melting of the northern and southern ice caps proceeds at the same rate, then we find that $J'_l(S) \approx (-1)^l J'_l(N)$ to within 20%. From Fig. 4, we can deduce that $J'_2(S) \approx J'_2(N)/2$, $J'_4(S) \gg J'_4(N)$ while the $l=3$ and $l=6$ contributions tend to cancel.

The present values of $\dot{J}_l(N)$ and $\dot{J}_l(S)$ as a function of the relaxation time τ_l are shown in Fig. 5. For $\dot{J}_2 = -3.5 \times 10^{-11} \text{ yr}^{-1}$, the allowed τ_2 are 3,100 or 65,000 yr which correspond to mantle viscosities of 4×10^{22} P or 8×10^{23} P, respectively¹¹. The remaining τ_l probably do not differ from τ_2 by more than a factor of two. Peltier³⁴ estimates that $\tau_5 \approx 2,500$ yr from the history of raised beaches in Hudson Bay.

If all τ_i are equal, then the values of τ_2 and $\dot{J}_2$ which account for Lageos' secular acceleration (12) is slightly changed to

$$\tau_2 = 2,800 \text{ yr}; \quad \dot{J}_2 = -3.1 \times 10^{-11} \text{ yr}^{-1} \quad (19)$$

$$\tau_2 = 75,000 \text{ yr}; \quad \dot{J}_2 = -3.2 \times 10^{-11} \text{ yr}^{-1} \quad (20)$$

The change is primarily due to the contribution to $\ddot{\Omega}(\text{LLR})$ from $\dot{J}_4$ since $\dot{J}_6$ is apparently small. Both $\dot{J}_2$ predict $(\dot{\omega}/\omega)_{\text{NT}} \approx 6 \times 10^{-11} \text{ yr}^{-1}$, in close agreement with the revised determination of Morrison and Stephenson⁹.

However, there are other considerations which affect the smaller τ_2 solution. The relaxation hypothesis also predicts that $(\dot{\omega}/\omega)_{\text{NT}}$ is not constant but decreases exponentially with time constant τ_2 . We would expect that the present $(\dot{\omega}/\omega)_{\text{NT}} \leq 4 \times 10^{-11} \text{ yr}^{-1}$ using $\tau_2 \leq 3,000 \text{ yr}$ and the 25-century average for $(\dot{\omega}/\omega)_{\text{NT}}$. The least-squares fit of post 1650 observations²⁹ suggest that the present $(\dot{\omega}/\omega)_{\text{NT}} \approx 17 \times 10^{-11} \text{ yr}^{-1}$. The discrepancy between the 3- and 25-century averages for $(\dot{\omega}/\omega)_{\text{NT}}$ is usually attributed to quasi-periodic exchanges of angular momentum between fluid core and mantle. Further analysis of the relaxation mechanism will be required to determine if there is a serious conflict between the historical $(\dot{\omega}/\omega)_{\text{NT}}$ and the $(\dot{\omega}(J_2)/\omega)_{\text{NT}}$ from Lageos with a 'small' τ_2 .

Seasonal variations in Ω

Comparison of the observed seasonal corrections with the nominal tidal coefficients of Table 2 indicates that the corrections are too large to attribute to errors in ocean tide models. The cause of the corrections is primarily nontidal J_2 variations ($\delta J_2(\text{annual}) \approx 3 \times 10^{-10}$ and $\delta J_2(\text{semiannual}) \approx 1 \times 10^{-10}$). The equivalent annual and semiannual amplitudes in $\delta \text{UT1}(J_2)$ are about 2.6 and 0.7 ms respectively. Each represents $\sim 10\%$ of the total seasonal variations in UT1 at those periods.

We are only partly successful in reconciling the observed annual and semiannual amplitudes in $\delta \Omega$ given in Table 2 with independent calculations of the nontidal seasonal contributions to δJ_2 and Earth rotation. The primary sources of nontidal seasonal δJ_2 are variations in groundwater, sea level and air mass. Table 2 also displays the predicted amplitudes based on the compilations of Munk and MacDonald¹³ and Lambeck¹⁴. Lambeck's estimate should be more accurate as he uses improved estimates of the groundwater variation¹⁶. There is a $\approx 45^\circ$ phase difference between the annual $\delta \Omega(\text{LLR})$ solution and Lambeck's prediction. Part of this discrepancy may be that the δJ_4 and δJ_6 contributions have been omitted from the model calculation. However, if these seasonal variations are primarily controlled by changes in mean sea level (a debatable point), then the expected scaling of the J_i is roughly³⁵; $\delta J_2 \approx -2\delta J_3 \approx 3\delta J_4 \approx -\delta J_5 \approx 4\delta J_6$, and δJ_2 clearly dominates.

In any case we have demonstrated that Lageos' orbit is sensitive to seasonal changes in the gravity field. We should also mention that the seasonal δJ_2 are expected to be noisy, varying in amplitude and phase from year to year and displaying spectral amplitude at all frequencies. How much of this seasonal 'noise' contributes to the residual amplitude in the $\delta \Omega(\text{LLR})$ spectra would be pure conjecture at present.

Future prospects

Our ability to separate the 18.6-yr and t^2 signatures should rapidly improve with time. Table 3 shows the expected uncertainties if we attempt to solve simultaneously for a $\sin \Omega_i$, $\cos \Omega_i$, and a quadratic polynomial in t as the data span is increased. These estimates assume that the background spectral amplitude

noise is $\sim 1.5 \text{ m arc s}$. At least $1\frac{1}{2}$ more years of data are required before the attempted separation of the long periodic and secular accelerations of Ω is justified, and as much as five additional years may be needed to obtain a strong separation.

Meanwhile, confirmation of our results may be possible from an independent analysis of the Starlette orbit. Starlette, launched on 1 February, 1975 (14 months before Lageos) is considerably more sensitive to variations in δJ_2 : $\delta \Omega(J_2) \approx 5 \delta \text{UT1}(J_2)$. Also the inclination parameter $f_4 = 0.04$ for Starlette is small enough to allow separation of δJ_2 and δJ_4 if δJ_6 is small. The major reservation is that Starlette's orbit is much more difficult to model numerically. Presently, the r.m.s. residuals to a fit of 1 yr of Starlette data is $\approx 8 \text{ m}$ compared with Lageos r.m.s. residuals of $\approx 2 \text{ m}$ for $5\frac{1}{2} \text{ yr}$ of data³.

Future improvements in the Lageos modelling may permit detection of seasonal and secular variations in the odd zonal J_i . In this case, the orbital eccentricity e and argument of perigee w are the orbital parameters most sensitive to changes in J_3 , J_5 , and so on. The dynamical equation appropriate to a high I , small e orbit is best described using the complex variable $p = e \exp(-iw)$ where $i = \sqrt{-1}$.

$$\frac{dp}{dt} + i\dot{w}_0 p \approx \frac{3}{2} n \sin I \left(\frac{R_e}{a} \right)^3 (\delta J_3 f_3 + \delta J_5 f_5) \quad (21)$$

$$f_3 = 1 - \frac{5}{4} \sin^2 I \quad (22)$$

$$f_5 = -\frac{5}{32} \left(\frac{R_e}{a} \right)^2 (8 - 28 \sin^2 I + 35 \sin^4 I) \quad (23)$$

$$\dot{w}_0 \approx 3 \left(\frac{R_e}{a} \right)^2 n J_2 f_3 \quad (24)$$

The inclination parameters equal $f_3 = -0.11$ and $f_5 = -0.45$ for Lageos while $\dot{w}_0 \approx -2\pi/1,707 \text{ days}$. The viscous rebound hypothesis predicts that $\dot{J}_3 \approx -1 \times 10^{-12} \text{ yr}^{-1}$ and $\dot{J}_5 \approx 1 \times 10^{-11} \text{ yr}^{-1}$ for $\tau_3 \approx \tau_5 \approx 2,500 \text{ yr}$. Therefore, the expected drift rate for p is $2 \text{ m arc s yr}^{-1}$. The seasonal δJ_5 may also dominate in equation (21). A plausible annual $\delta J_5 \approx 2 \times 10^{-10}$ in amplitude, will produce a 9 m arc s annual variation in p . Both of these signatures should be detectable from the present data record. The major reservation here is that variable drag forces³⁶ and seasonal and irregular variations in the Earth light pressure force may produce similar signatures and are not yet adequately modelled. An improved model for the light pressure force is being developed.

Conclusion

We have demonstrated that Lageos' node is sensitive to seasonal and secular changes in the Earth's gravity field, especially $\dot{J}_2$. The observed secular acceleration $\delta \Omega(\text{LLR}) = -14.7 \pm 1.3 \text{ m arc s yr}^{-2}$ can be driven by a secular change in J_2 of $-3 \times 10^{-11} \text{ yr}^{-1}$. This value for $\dot{J}_2$ includes corrections from $\dot{J}_4$ and $\dot{J}_6$ predicted by mantle relaxation following deglaciation [Fig. 4 and equation (18)], for a mantle viscosity near $3 \times 10^{22} \text{ P}$. Any dissipation in the 18.6-yr tide will reduce the derived $\dot{J}_2$ further. This $\dot{J}_2$ predicts a modern value for the nontidal acceleration of the Earth's rotation $(\dot{\omega}/\omega)_{\text{NT}} \approx 6 \times 10^{-11} \text{ yr}^{-1}$. An independent analysis of Starlette's orbit might confirm this prediction for $\dot{J}_2$.

Annual and semiannual signatures in Ω have been detected and agree in amplitude, but not in phase with independent estimates of the contributions to δJ_2 and Earth rotation from changes in groundwater, sea level and air mass. There must be improvements in the seasonal model before we say that this discrepancy is significant. The inferred UT1 (δJ_2) variations are $\sim 10\%$ of the known seasonal terms.

In the future, it should be possible to detect similar variations in the odd J_i from their effect on Lageos' eccentricity. With five more years of Lageos' data, we should also be able to detect the effects of dissipation on the 18.6-yr ocean tide if the $Q(18.6 \text{ yr}) \leq 50$.

Finally, we have indirectly demonstrated that UT1(LLR) has better long term stability (spectral period $\geq 60 \text{ days}$) than does UT1(BIH) from a comparison of the post-fit amplitude

Table 3 Estimated uncertainties as function of data span

Data span (yr)	$\sin \Omega_i$ (m arc s)	$\cos \Omega_i$ (m arc s)	$(t^2 \text{ yr}^{-2})$ (m arc s)
$5\frac{1}{2}$	± 300	$\pm 1,400$	± 80
7	90	160	10
8	60	80	5
10	30	20	1.7
12	20	10	1.1

spectrum of the residual $\delta\Omega$ using both sources of UT1. While Lageos may determine short-period variations in UT1 well, at longer times scales its unique sensitivity to changes in the zonal harmonics will be more valuable than its direct sensitivity to UT1 since this permits isolation of the component of UT1 variations caused by J_2 changes.

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Constraints on evolution of Earth's mantle from rare gas systematics

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Analyses of the isotopic composition of He, Ar and Xe in a suite of glasses from the mid-ocean ridges and from the island of Hawaii show that the Hawaiian samples have systematically lower $^4\text{He}/^3\text{He}$, $^{40}\text{Ar}/^{36}\text{Ar}$ and $^{129}\text{Xe}/^{130}\text{Xe}$ ratios than the mid-ocean ridge basalts. We interpret this result to imply the existence of an undegassed mantle reservoir. Given the isotopic variations, and the half lives of ^{129}I and ^{40}K (parent isotopes of ^{129}Xe and ^{40}Ar), the undegassed reservoir must have been separated from the MORB source reservoir at least 4,400 Myr ago. The most reasonable explanation for the data is therefore the existence of a two-layered mantle.

THE search for the driving mechanism of plate tectonics has led geophysicists to propose a series of convective mantle models. Morgan¹ proposed the existence of a separate deep mantle, which would generate plumes. McKenzie and Richter² proposed a two-layer convection mantle, with a boundary layer at 700 km (maximum depth of earthquakes). On the other hand, several authors^{3–5} have argued that geophysical evidence favours whole-mantle convection.

Measurements of isotope ratios in mantle-derived basalts have shown that the mantle is heterogeneous. Based on Sr and Nd isotopic information alone, early studies showed that distinction could be made between a depleted mantle source (that is, the source of mid-ocean ridge basalts (MORB)), and an undepleted mantle, from which oceanic island basalts are derived (see reviews in ref. 6). Assuming that depletion is caused by the extraction of continental crust, and using the geochemical budgets for Sr and Nd, several authors^{7–10} calculated that the depleted mantle represents one-third or half of the whole mantle.

Reconciling this conclusion with a two-layered mantle model, these authors assume that the upper 700 km correspond to the depleted mantle. There are several major criticisms of this model. First, it is not clear that depletion is created only by extraction of continental crust, since basaltic extraction on the ridge crest may also have an important role^{7,11}. Second, the geochemical budget calculations do not yield the relative geometry of the two assumed mantle reservoirs. Finally, the

assumption of only two geochemically homogeneous distinct reservoirs is an oversimplification^{10,12,13}.

Nd-Sr isotope geochemistry does not allow a unique choice to be made between whole-mantle and layered-mantle convection. For example, proponents of whole-mantle convection have argued that the geochemical heterogeneities can be explained by a lumpy mantle, rather than a layered one (see ref. 14). This would require that the lumps maintain their identity over long periods of time, and are entrained in the overall circulation pattern.

We now report new noble gas measurements on oceanic basalt glasses, and illustrate the importance of these data to the above arguments. Although in recent years several noble gas studies have been carried out on mantle derived rocks (see refs 12, 15–21) this information has not received the attention that it deserves. The noble gases can yield unique constraints on the structure and evolution at the mantle for several reasons. (1) Due to the volatility of the noble gases, and the involatility of the radioactive parent isotopes (^{40}K , ^{238}U , ^{235}U , ^{232}Th , ^{244}Pu), the isotope evolution is strongly affected by degassing, which is not the case for the Sm-Nd and Rb-Sr systems. Therefore, the variation of mantle noble gas isotope ratios depends on the degassing history of the mantle, and are a function of continental extraction processes only through their parent isotopes.

(2) The radioactive parent isotopes decay with very different half lives which provides unique temporal information. The extinct isotopes ^{129}I and ^{244}Pu had very fast decay constants

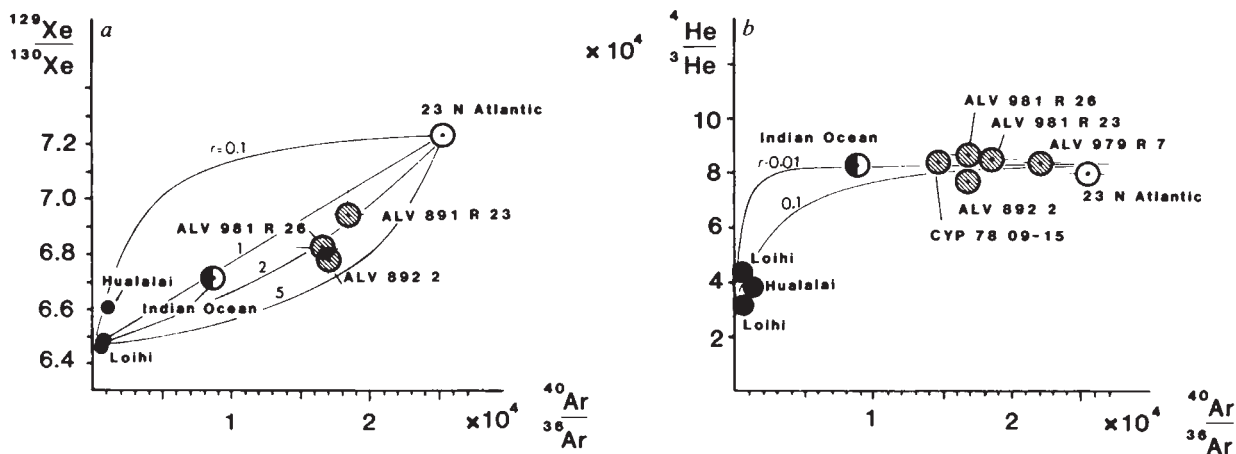


Fig. 1 *a*, $^{129}\text{Xe}/^{130}\text{Xe}$ versus $^{40}\text{Ar}/^{36}\text{Ar}$ for the oceanic basalt glass data given in Tables 1 and 2. For sample ALV 979 R7 no xenon data were measured. Sample CYP 78-09-15 is not reported because of possible atmospheric contamination. *b*, $^4\text{He}/^3\text{He}$ versus $^{40}\text{Ar}/^{36}\text{Ar}$ for the same samples as in *a*. Note that the points retain the same relative positions on the two diagrams. *r* stands for the ratio $(^{130}\text{Xe}/^{36}\text{Ar})_{\text{LOIHI}}/(^{130}\text{Xe}/^{36}\text{Ar})_{\text{MORB}}$ and $(^3\text{He}/^{36}\text{Ar})_{\text{LOIHI}}/(^3\text{He}/^{36}\text{Ar})_{\text{MORB}}$ in *a* and *b* respectively. It characterizes the curvature of the hyperbolic mixing curves (see ref. 33). Note that contamination of MORB with air in *a* would result in a curve with $r=0.1$. For sample description and locations see: AII 96-18-1 in ref. 42 and kk 9-14 in ref. 31; kk-24-7 and kk-29-10 in ref. 39; ALV 892-2 in ref. 16; CYP 78 09-15 in ref. 40; ALV 981 R23 & R26 in ref. 41; ALV 979 R7 is from $105^\circ 05' 50'' \text{W}$, $20^\circ 50' 10'' \text{N}$; MD23 DR04 from $25^\circ 52' \text{S}$, $69^\circ 19' \text{E}$, west indian ridge (R. Schlich, personal communication).

($4.36 \times 10^{-8} \text{ yr}^{-1}$ and $8.4 \times 10^{-9} \text{ yr}^{-1}$, respectively) and therefore, xenon isotopic anomalies with respect to their daughter isotopes are very old, having been produced in the first 100 Myr of Earth history. On the other hand, spontaneous fission of ^{238}U is much slower ($\lambda_{\text{sf}} = 8.47 \times 10^{-17} \text{ yr}^{-1}$; $\lambda_{\alpha} = 1.54 \times 10^{-10} \text{ yr}^{-1}$), has occurred throughout the age of the Earth, and produces a different xenon isotope signature than decay of ^{244}Pu (refs 22-24). ^{40}Ar is produced by decay of ^{40}K ($\lambda = 5.543 \times 10^{-10} \text{ yr}^{-1}$), and ^4He is produced by decay of ^{238}U , ^{235}U , and ^{232}Th .

(3) The different rare gases span a large range of atomic number and atomic size, and may have quite different mantle transport properties.

Rare gas isotope variations in basalts

A recent study of xenon isotopes in MORB glasses¹⁷ documented a large anomaly in $^{129}\text{Xe}/^{130}\text{Xe}$ ratio with respect to the atmosphere. Due to the short half life of extinct ^{129}I , this result clearly shows that the separation between the MORB mantle source and the atmosphere occurred 4,400 Myr ago. While this conclusion requires few assumptions, the data did not allow an evaluation of the xenon isotopic signature of the whole mantle, since the MORB mantle is only a fraction of the total. Initially, we interpreted the variations within the suite of MORB glasses to reflect atmospheric contamination¹⁷. However, there is an alternative explanation, namely, that a significant volume of the mantle contains xenon of atmospheric isotopic composition. The challenge to the experimentalist is to distinguish between these two possibilities.

A similar situation exists for argon isotopes. The literature contains reported $^{40}\text{Ar}/^{36}\text{Ar}$ ratios for igneous rocks between atmospheric 295.5 and 16,000 (refs 25-27). The importance of the high MORB $^{40}\text{Ar}/^{36}\text{Ar}$ ratios to degassing models is explained in ref. 18. The controversy regarding interpretation of the low $^{40}\text{Ar}/^{36}\text{Ar}$ ratios is an old one^{28,29}; the present consensus seems to be that most are the result of contamination, either in the laboratory or by interaction with atmosphere during emplacement (see ref. 30).

In contrast, helium isotopic studies of igneous rocks are not subject to equivalent problems of contamination because helium is the least adsorptive of gases and is present only in trace quantities in air. The detection of excess ^3He (relative to air) in MORB glasses can only be interpreted as evidence for the existence of 'primordial' mantle helium^{15,16}. The large variations in $^4\text{He}/^3\text{He}$ ratios observed for oceanic basalts^{12,32} therefore are not the result of atmospheric contamination, but must

reflect mantle heterogeneity. The samples with the highest excess of ^3He (from Hawaii¹²) can therefore be identified as derived from the most undegassed mantle. Although helium isotope ratios are often reported as $^3\text{He}/^4\text{He}$, we tabulate them here as $^4\text{He}/^3\text{He}$ to conform with the convention for other radiogenic isotope systems (that is radiogenic isotope/stable isotope).

The strategy employed in this study was to use the helium isotopes to identify samples from normal MORB type mantle and from undegassed mantle^{12,21}. The goal was to document the differences in argon and xenon isotopic compositions between these two different mantle 'types'. Given the controversy regarding atmospheric contamination, several special precautions were taken. All analyses were performed on fresh vitreous glasses which were hand-picked to avoid alteration, and sonically cleaned in ethanol. To minimize the effective surface area available for adsorption, and gas loss from the vesicles¹⁶, only large chunks of glass (between 3 and 10 mm size) were analysed. To eliminate any further question of atmospheric contamination, stepwise heating was applied to all the samples. Any atmospheric gases not removed during the pre-analysis bake-out (200°C) should be released in the low-temperature step. Finally, blanks were run before and after each sample. As the procedure was identical for all samples, isotopic differences cannot be attributed to atmospheric contamination.

The results for three basaltic glasses from the island of Hawaii and seven MORB glasses are presented in Table 1. Experimental details are given in refs 17, 18. Helium isotopic analyses of the Hawaiian samples have been reported previously³¹ as have two of the MORB samples (AII 96-18-1 and ALV 892-2). Although the $^4\text{He}/^3\text{He}$ ratios presented here were obtained on a glass mass spectrometer optimized for the heavy noble gases, reasonable agreement is obtained (within 15%), considering the larger uncertainty (see refs 16, 31).

The results presented in Table 1 reveal important differences between the MORB and Hawaiian samples. The MORB glasses are characterized by typical $^4\text{He}/^3\text{He}$ ratios of $1/9 \times$ atmospheric and quite high $^{40}\text{Ar}/^{36}\text{Ar}$, and $^{129}\text{Xe}/^{130}\text{Xe}$ ratios, relative to atmospheric. The sample AII 96-18-1 has the highest $^{129}\text{Xe}/^{130}\text{Xe}$ and $^{40}\text{Ar}/^{36}\text{Ar}$ ratios ever reported for a MORB glass ($25,250 \pm 680$ and 7.23 ± 0.4 respectively). In contrast, the Hawaiian samples (from Loihi Seamount and Hualalai) have lower $^4\text{He}/^3\text{He}$ ratios (see also ref. 31) and lower $^{40}\text{Ar}/^{36}\text{Ar}$ and $^{129}\text{Xe}/^{130}\text{Xe}$ ratios. The $^{40}\text{Ar}/^{36}\text{Ar}$ ratio for the sample with the lowest $^4\text{He}/^3\text{He}$ ratio (kk 29-10 from Loihi

Table 1 Helium and argon data for glassy samples from Hawaii and mid-oceanic ridges

Sample	Temperature (°C)	^3He ($10^{-12} \text{ cm}^3 \text{ g}^{-1}$)	$^4\text{He}/^3\text{He}$	^{36}Ar ($10^{-10} \text{ cm}^3 \text{ g}^{-1}$)	$^{40}\text{Ar}/^{36}\text{Ar}$
Loihi					
KK-29-10	700	4.55	34,670±8,130	3.64±0.03	305±5
	1,000	28.77	30,810±1,980	7.03±0.06	425±8
	1,450	5.05	32,230±2,460	1.56±0.02	447±5
	1,650	—	—	0.086±0.006	379±5
	Total	38.37	31,460±1,760	12.32±0.07	392±5
KK-24-7	700	2.51	37,690±2,030	3.5±0.1	308±16
	1,000	1.98	41,180±4,770	8.3±0.3	385±15
	1,400	0.39	101,200±24,160	35.1±0.4	336±6
	1,650	—	—	112.0±2.0	300±5
	Total	4.88	42,880±2,390	158.9±2.06	313±4
Hualalai					
KK-9-14	700	0.26	251,300±45,400	5.11±0.13	377±12
	1,400	43.61	36,960±203	406.2±4.2	1,150±19
	1,650	—	—	2.01±1.25	2,900±1,830
	Total	43.87	38,210±320	413.3±4.38	1,149±19
Atlantic					
AII 96-18-1	700	12.01	81,700±1,770	0.037±0.006	23,810±3,520
	1,300	183.49	79,110±260	0.420±0.010	25,250±690
	1,650	—	—	0.051±0.022	18,300±820
	Total	195.50	79,270±240	0.508±0.025	24,450±1,210
Indian Ocean					
MD23 DR04	1,150	329.61	81,730±330	1.33±0.02	8,660±80
	1,400	131.26	84,750±920	1.04±0.03	7,040±300
	1,650	—	—	—	—
	Total	460.87	82,680±350	2.37±0.04	7,960±150
Pacific					
ALV 981 R 23	600	10.13	95,200±1,320	0.235±0.012	295±15
	850	37.53	90,890±510	0.187±0.102	350±175
	1,000	67.94	76,950±1,110	0.233±0.010	5,540±240
	1,150	12.37	84,710±610	0.128±0.017	16,320±2,160
	1,400	19.71	90,090±2,100	0.210±0.024	18,260±2,040
	1,500	—	—	0.130±0.035	1,500±500
	Total	147.68	84,150±1,420	1.123±0.113	6,720±670
ALV 981 R 26	1,000	81.35	80,640±530	0.245±0.015	3,270±180
	1,400	26.63	101,060±2,850	0.217±0.020	16,600±2,500
	1,600	—	—	0.163±0.048	15,300±5,800
	Total	107.98	85,680±1,450	0.625±0.054	10,890±1,010
ALV 979 R 7	950	41.96	81,520±2,210	0.045±0.009	930±190
	1,300	95.53	83,840±390	0.242±0.017	21,890±1,530
	1,650	—	—	0.148±0.022	14,480±2,150
	Total	137.49	83,130±740	0.435±0.029	17,190±1,150
CYP 78 09-15	950	238.86	83,130±260	0.98±0.012	14,500±300
	1,650	120.17	82,810±350	2.23±0.07	3,660±185
	Total	359.03	83,030±210	3.21±0.07	6,970±190
Galapagos Rise					
ALV 892-2	700	1.40	67,810±4,700	0.0155±0.0005	329±14
	900	43.14	75,220±2,920	0.074±0.002	14,870±500
	1,200	3.31	88,130±3,380	0.077±0.002	16,680±38 ^c
	1,400	15.86	74,850±2,410	0.082±0.002	12,920±290
	1,650	11.21	79,020±4,870	0.122±0.014	14,550±1,640
	Total	35.26	76,150±1,910	0.370±0.014	14,120±540
Atmosphere			722,540		295.5

All samples have been degassed in several temperature steps. Errors are $2\sigma/\sqrt{N}$. Absolute concentrations are uncertain to $\pm 10\%$. The sensitivity for He of $2.7 \times 10^{-11} \text{ cm}^3 \text{ mV}^{-1}$ is calculated by comparison with samples ALV 892-2 and AII 96-18-1 measured by Kurz³² and differs from the value used previously¹⁷.

Seamount) is only slightly above atmospheric, and the xenon isotopic composition is indistinguishable from air. The quantities of argon and xenon are both well above the blanks, which were less than 8%, and 10% respectively in all cases.

These isotopic variations are shown on the $^4\text{He}/^3\text{He}$ versus $^{40}\text{Ar}/^{36}\text{Ar}$ and $^{129}\text{Xe}/^{130}\text{Xe}$ versus $^{40}\text{Ar}/^{36}\text{Ar}$ diagrams in Fig. 1. The correlation between $^{129}\text{Xe}/^{130}\text{Xe}$ and $^{40}\text{Ar}/^{36}\text{Ar}$ strongly suggests that atmospheric or seawater contamination cannot be responsible for the variations. Based on the $^{130}\text{Xe}/^{36}\text{Ar}$ ratios in the glass samples and in air, any mixing line between MORB and atmosphere should be convex on Fig. 1 and is represented by $r = 0.1$ (see refs 33, 34 for a discussion of ratio-ratio plots).

Atmospheric contamination also could not explain the striking negative correlation between $^{40}\text{Ar}/^{36}\text{Ar}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ (Fig. 2).

Interpretation

It is generally assumed that mantle gases are residual from atmosphere formation. The noble gas isotopic ratios of the MORB glass samples presented here and elsewhere^{17,18} are consistent with this idea, and suggest that the mantle source of MORB was degassed early in Earth's history. The isotopic ratios of the radiogenic or fissiogenic noble gases depend on the μ value for the isotope system (that is, the radioactive parent/reference isotope ratios: U,Th/ ^3He , $^{40}\text{K}/^{36}\text{Ar}$,

$^{129}\text{I}/^{130}\text{Xe}$), and the mantle degassing history. Therefore, the very high radiogenic daughter/reference isotope ratios ($^4\text{He}/^3\text{He}$, $^{40}\text{Ar}/^{36}\text{Ar}$, $^{129}\text{Xe}/^{130}\text{Xe}$) for MORB are the result of early outgassing^{17,18}.

The results for the Loihi seamount and Hualalai samples require an alternative explanation. Based on the Xe–Ar correlation, the Ar–Sr correlation, the helium isotopic results^{12,29}, and our experimental precautions, we can immediately eliminate surficial contamination processes. We are therefore left with two hypotheses: the lower $^{40}\text{Ar}/^{36}\text{Ar}$ and $^{129}\text{Xe}/^{130}\text{Xe}$ ratios (close to atmosphere) are either the result of recycled atmospheric gases in the mantle, or the presence of an undegassed mantle reservoir which has retained its noble gases over the age of the Earth. For these samples, the recycled atmospheric gas hypothesis is not likely because any reasonable recycling mechanism (such as subduction of oceanic crust/sediments) would enrich the mantle in U and K, relative to the rare gases, and produce, after a certain time span, considerable amounts of radiogenic noble gases, leading to higher $^4\text{He}/^3\text{He}$ and $^{40}\text{Ar}/^{36}\text{Ar}$ ratios; neither of which is observed. Therefore, we conclude that the mantle source for the Loihi and Hualalai samples is less degassed than the MORB source, and contains noble gas isotope ratios similar to the atmosphere (except for helium, due to atmospheric escape).

Similar conclusions have been reached by other workers based on different information. Kaneoka and Takaoka²¹ noted a correlation between $^3\text{He}/^4\text{He}$ and $^{40}\text{Ar}/^{36}\text{Ar}$ qualitatively similar to the one displayed in Fig. 1b. However, they observed no correlation with respect to xenon, and analysed phenocrysts and xenocrysts rather than glasses. Based on a literature search, Hart *et al.*³⁵ also suggested the existence of undegassed mantle with low $^{40}\text{Ar}/^{36}\text{Ar}$ ratios. Manuel and Sabu³⁶ suggested similar conclusions for xenon. The present study offers compelling evidence for an undegassed mantle reservoir, particularly since all the noble gases were measured on the same samples, all of which were fresh glasses.

This has quite important implications for models of mantle structure and evolution. As mentioned earlier, the short half life of extinct ^{129}I (parent of ^{129}Xe) means that any differences in $^{129}\text{Xe}/^{130}\text{Xe}$ ratio within the mantle must be at least 4,400 Myr old. Using the $^{129}\text{Xe}/^{130}\text{Xe}$ isotopic differences between MORB and the atmosphere, Staudacher and Allègre¹⁷ calculated that these two systems were separated 10–25 Myr after accretion of the Earth. The calculation is analogous to the separation between the mantle sources of MORB and Hawaiian basalts, since the difference in $^{129}\text{Xe}/^{130}\text{Xe}$ ratio is similar. Therefore, any mantle model must allow the existence of distinct reservoirs for at least 4,400 Myr. This conclusion is one of the strongest arguments yet offered in favour of a layered mantle. In view of the homogenizing effect of convection (see ref. 37), whole mantle convection over the age of the Earth would have destroyed such heterogeneities in the xenon isotopes, and is therefore untenable. Similar conclusions are reached from the argon isotopes, although the mean ages are slightly higher due

to the longer half life of ^{40}K , and the greater effect of recycling on argon¹⁸.

Note that the relation between degassing and depletion (as used in reference to Rb/Sr, Sm/Nd) is not necessarily simple. Although the noble gases suggest that the separation between the two mantle layers (MORB and Hawaiian sources) and the atmosphere occurred at least 4,400 Myr ago, Sr and Nd isotopic variations were produced by extraction of the continents, which proceeded later and at a different rate^{7,38}. To the extent that isotope ratio variations are generated by present day (recent) mantle mixing, correlations should be observed between the noble gases and the isotopes of Sr and Nd. In fact, Kurz *et al.* observed correlations between $^3\text{He}/^4\text{He}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ on both regional and global scales. The results presented here support the hypothesis that mixing is responsible for the isotopic variations, as is shown by the correlation between $^{40}\text{Ar}/^{36}\text{Ar}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ in Fig. 2. The systematics suggest that the mantle source for MORB is both depleted with respect to Rb/Sr and degassed with respect to noble gases, which is also consistent with a layered mantle. Even though degassing and continent formation may not have occurred at the same time, they apparently both acted on the mantle source of MORB, which is presumed to be the upper mantle.

These systematics do not rule out more complex descriptions of the mantle, involving heterogeneities within each of the layers. As mentioned earlier, isotopic evidence suggests that more than two discrete 'reservoirs' are required¹², possibly including recycled oceanic crust, and mantle that is affected by core-pumping⁷. The samples analysed for this study were chosen specifically to delineate the first-order effects between the best representatives of depleted and undegassed mantle. Note also

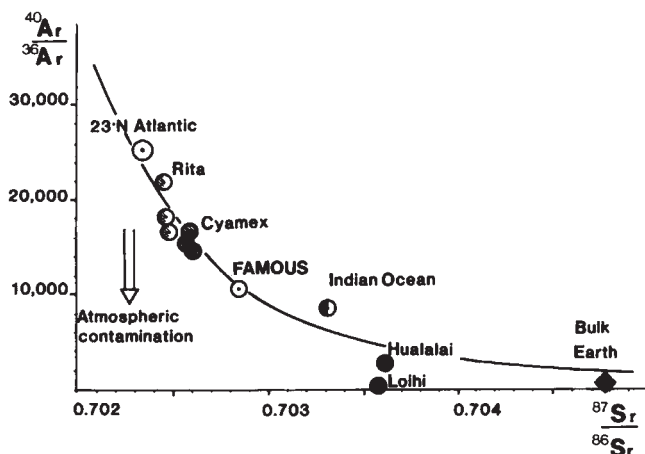


Fig. 2 $^{40}\text{Ar}/^{36}\text{Ar}$ versus $^{87}\text{Sr}/^{86}\text{Sr}$ for the oceanic basalt glasses. Sources for values of $^{87}\text{Sr}/^{86}\text{Sr}$: Loihi seamount from ref. 12, Hualalai, D. Clague in ref. 31; North Atlantic sample AII 96 18-1 from ref. 42. For all other samples only the argon isotopic composition was measured. The strontium values and argon data are given in ref. 18.

Fig. 3 A simplified mantle profile showing possible differences between depleted and degassed mantle reservoirs. Since the degassing took place 4,400 Myr ago (see text), and continent formation did not occur until much later, this figure illustrates one possible complication that this different timing may imply.

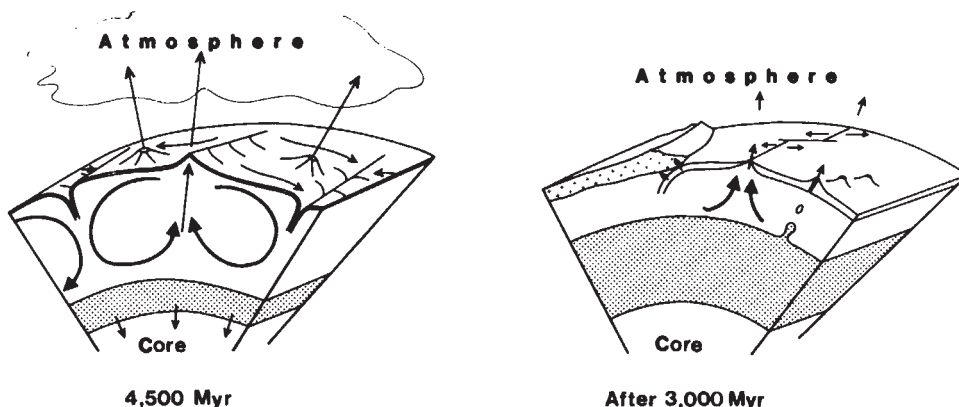


Table 2 Xenon isotopic data normalized to $^{130}\text{Xe} = 100$

Sample	Temperature (°C)	^{130}Xe (10^{-14} $\text{cm}^3 \text{g}^{-1}$)	^{124}Xe	^{126}Xe	^{128}Xe	^{129}Xe	^{130}Xe	^{131}Xe	^{132}Xe	^{134}Xe	^{136}Xe
Loihi											
KK-29-10	700	7.08±0.13	—	—	50.3±1.9	661±10	100	522±4	664±7	262±2	212±2
	1,000	16.26±0.11	1.9±0.4	2.2±0.5	46.8±0.9	648±5	100	515±4	658±7	257±2	216±2
	1,400	2.60±0.06	—	2.3±3.3	46.3±2.1	636±7	100	505±5	646±15	259±3	215±3
	1,650	<0.70	—	—	—	—	—	—	—	—	—
KK-24-7	700	<0.40	—	—	—	—	—	—	—	—	—
	1,000	13.3±0.13	—	—	47.5±0.6	644±7	100	520±5	663±6	256±3	221±2
	1,400	6.3±0.06	—	—	43.9±0.9	647±5	100	517±3	664±3	261±2	221±2
	1,650	8.1±0.12	—	—	45.9±0.5	644±3	100	523±2	662±4	259±2	222±1
Hualalai											
KK-9-14	700	<0.15	—	—	—	—	—	—	—	—	—
	1,400	11.6±0.14	2.2±0.8	2.7±0.9	45.5±1.0	660±2	100	525±2	659±3	261±1	222±1
	1,650	<0.2	—	—	—	—	—	—	—	—	—
Atlantic											
AII 96-18-1	700	0.5±0.2	—	—	—	—	—	—	—	—	—
	1,300	4.11±0.06	3.0±1.3	3.7±1.4	45.9±1.5	723±4	100	520±3	666±9	276±2	238±2
	1,650	1.30±0.10	6.6±2.8	—	39.7±11.9	689±21	100	520±16	673±31	266±10	227±9
Indian Ocean											
MD23 DR04	1,150	10.90±0.03	—	2.25±0.07	45.6±0.2	671±2	100	519±2	659±2	263±1	224±1
	1,400	14.50±0.04	—	2.05±0.09	46.9±0.2	671±2	100	482±2	662±2	260±1	223±1
	1,650	4.31±0.06	—	2.44±0.43	44.4±1.3	659±9	100	524±7	664±9	260±4	223±2
Pacific											
ALV981 R 23	600–1,150	—	—	—	—	—	—	—	—	—	—
	1,400	2.3±0.3	—	—	—	694±11	100	503±8	649±9	265±5	238±3
	1,500	—	—	—	—	—	—	—	—	—	—
ALV981 R26	1,000	1.02±0.3	—	—	59±7	633±18	100	555±19	633±17	244±9	208±7
	1,400	2.60±0.3	—	—	47±1	681±7	100	521±6	662±7	266±3	224±2
	1,600	2.44±0.4	—	—	49±2	680±12	100	521±9	658±11	265±5	223±4
Galapagos Rise											
ALV 892-2	700	<0.2	—	—	—	—	—	—	—	—	—
	900	1.3±0.1	—	—	48.4±7.7	606±43	100	484±35	607±43	240±18	228±18
	1,200	8.15±0.17	—	—	48.6±2.1	679±7	100	518±5	665±6	264±3	228±3
	1,400	9.22±0.38	—	—	50.2±2.7	670±11	100	505±9	642±12	260±5	222±4
	1,650	13.22±0.37	—	—	48.0±1.8	672±6	100	519±5	654±8	262±3	222±2
Atmosphere			2.35	2.21	47.0	648	100	519	659	256	217

that the correlations in Figs 1 and 2 do not necessary prove a one-to-one relation between degassed and depleted mantle sources. In fact, it is quite reasonable to assume that the convective structure of the mantle has changed since the degassing took place 4,400 Myr ago. One example of the complexity this may impart to present-day geochemical structure is shown in Fig. 3. If the depth of the top convecting layer decreased after degassing, the continents may then have been extracted from only a part of the degassed mantle, resulting in the existence of a degassed but undepleted reservoir (see Fig. 3). This is only one of the possibilities, which present a challenge both to geophysicists and geochemists.

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Conclusions

The isotopic composition of He, Ar, and Xe in several basalt glasses, from both mid-ocean ridges and the island of Hawaii, require the existence of both a degassed and an undegassed mantle reservoir. The isotopic variations of Ar and Xe, combined with the half lives of ^{40}K and ^{129}I , show that these two reservoirs must have been separated for at least 4,400 Myr, a conclusion which argues strongly for a layered mantle.

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'Hormonal' risk factors, 'breast tissue age' and the age-incidence of breast cancer

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For most cancer sites there is a linear log-log relationship between incidence and age. This relationship does not hold for breast cancer, and certain 'key' breast cancer risk factors suggest that breast tissue does not 'age' in step with calendar time. A quantitative description of 'breast tissue age' is suggested which brings the age-incidence curve of breast cancer into line with the common log-log cancers and explains quantitatively the known key risk factors. The model also explains the 'anomalous' finding that although early first birth is protective, late first birth carries a higher risk than nulliparity. US breast cancer rates are some four to six times the rates in Japan—the model suggests that the key risk factors, when considered jointly with weight, can explain about 85% of the difference.

EARLY menarche, late first full-term pregnancy (FFTP) and late menopause¹⁻⁵ are generally accepted as the three major risk factors for breast cancer⁶. The higher breast cancer risk among women with an earlier menarche^{2,4,5} is most noticeable at young ages, and we found a twofold increased risk for menarche at 11 yr as compared with menarche at 13 yr in cases under age 33⁵. With regard to late FFTP, MacMahon *et al.*^{1,4} in their international case-control study found that women with a FFTP under age 20 had about one-half the risk of nulliparous women, but that nulliparous women did not have as high a risk as women whose FFTP was after age 35. Single women and nulliparous married women had the same risk. Finally, Trichopoulos *et al.*³ estimated that women whose natural menopause (last menstrual period) occurred before age 45 had only one-half the risk of women whose menopause occurred after age 55. They also demonstrated the same effect with bilateral oophorectomy. We present here a simple statistical model of breast cancer incidence rates which incorporates these risk factors and puts the age-incidence pattern of breast cancer into context with non-hormone-dependent cancers. The model explains the 'anomalous' finding that late age at FFTP carries a higher breast cancer risk than nulliparity^{1,4} and emphasizes the role of hormonal stimulation of breast tissue as probably the critical factor in breast cancer pathogenesis.

Postmenopausal weight has recently been established as a risk factor. de Waard *et al.*⁷ found a relative risk of 1.62 when they compared women weighing 75 kg with women weighing 55 kg, and their results have been confirmed in the large American Cancer Society cohort study⁸. Our model does not initially take this factor into account, but it is an essential key to understanding the low breast cancer rates in Japan (see 'Discussion').

Age and cancer incidence

The incidence of most common non-hormone-dependent cancers shows a steady increase with age, which can be represented as

$$I(t) = at^k \quad (1)$$

where $I(t)$ is the probability of being diagnosed with the particular cancer within a year at age t (ref. 9). For these cancers a straight line is obtained if the logarithm of the incidence is plotted against the logarithm of age: k , the slope of the line, is usually between 4 and 5 (ref. 9).

Studies of lung cancer and cigarette smoking, including the effects of stopping smoking, showed that incidence should be related to duration of smoking rather than to age^{10,11} and suggest that we write equation (1) as

$$I(t) = a[d(t)]^k \quad (2)$$

where $d(t)$ is the 'relevant age' of the tissue. For lung cancer rates at a given cigarette consumption, $d(t)$ is simply duration of smoking to age t (minus some minimum 'latent period').

Age incidence of breast cancer

Because equation (2) has been so successful in describing the incidence rates of many cancers, it is reasonable to hope that it will be able to represent the incidence curve for breast cancer (Fig. 1) with $d(t)$ appropriately defined. A simple definition incorporating menarche, FFTP and menopause assumes that 'breast tissue ageing' starts at menarche, moves regularly (at rate $f_0 = 1$) to FFTP, then slows to rate f_1 until menopause, when it slows further to rate f_2 .

Although this definition of $d(t)$, with k set at 4.5 to agree with the best value of k for lung cancer incidence data¹¹, can be made to give a good fit¹² to the 1969-71 Third National Cancer Survey (TNCS) breast cancer incidence rates for US white females¹³, the definition does not account for a FFTP after age 35 being associated with a cancer rate that is larger than that of nulliparity^{1,4}. We can accommodate this fact by incorporating a constant, b , to represent a one-time increase in $d(t)$ associated with FFTP. With this definition of $d(t)$ we can mimic the effects of menarche and FFTP, but we predict too great an effect of menopause.

If we further modify $d(t)$ by incorporating a decline in the rate of breast tissue ageing starting at age 40 and ending at the

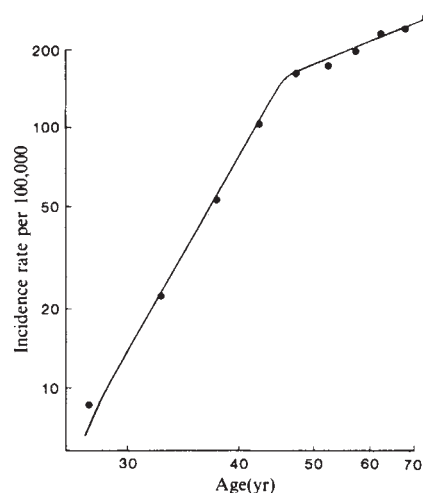


Fig. 1 Age-specific incidence rates for breast cancer in US white females (TNCS 1969-71)¹⁴ and fitted curve from model shown in Fig. 2.

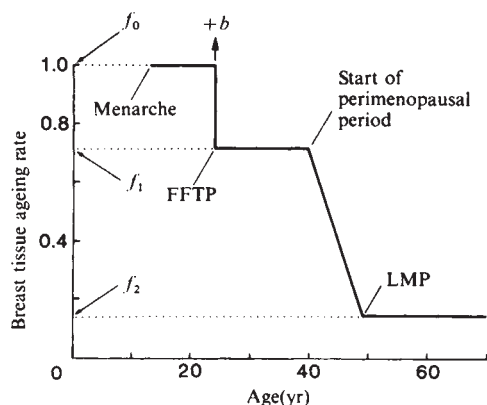


Fig. 2 Model of rate of breast tissue ageing (see text). LMP, last menstrual period.

last menstrual period (Fig. 2), the observed effects of menopause may also be accurately reproduced (see below). This modification agrees broadly with the data on hormonal profiles¹⁴ and menstrual cycle length¹⁵ of women in this age range.

Fitting model parameters

The parameters (f_1 , f_2 , b) of the model (Fig. 2) were estimated by comparing the predictions of the model with the breast cancer incidence rates of the TNCS for white women and with the results of various case-control studies.

The TNCS rates are cross-sectional, so that, in order to fit the model it was necessary to assess secular trends in age at menarche, FFTP and menopause. The secular trend in mean age at menarche was investigated using the data of MacMahon¹⁶, and may be expressed as mean age at menarche (yr) of cohort born in year $x = 13.88 - 0.01873(x - 1900)$. The distribution is adequately represented by a logistic curve $\text{Prob}(\text{menarche occurs at or before age } x) = \exp\{b(x - m)\} / [1 + \exp\{b(x - m)\}]$, where m is the mean age at menarche and b is 1.44 (ref. 16).

The distributions of age at first live-birth were obtained from fertility tables¹⁷. The distribution of FFTP is closely approximated by this distribution¹⁸.

Analysis shows that the age at natural menopause can be represented by a logistic function with mean 50.0 and slope 0.414 (refs 19, 20). We estimate that approximately 50% of hysterectomies are accompanied by bilateral oophorectomy^{21,22}, and using this value together with age-specific hysterectomy rates, the distribution of age at menopause (natural menopause or bilateral oophorectomy) may be estimated²⁰; it has a median of 48.3. There has been little secular change during the period relevant to our fitting the TNCS rates¹⁹.

To fit the model parameters, the total absolute relative deviation was chosen as the criterion for discrepancy between the predicted and observed rates (or relative risks).

We obtained initial estimates of f_1 and b by comparing observed and predicted relative risks associated with FFTP. This procedure was chosen because the magnitude of the effect of FFTP on breast cancer risk has been found to be similar across several studies^{1,4} and f_1 and b essentially determine the relative risks associated with FFTP. The data of MacMahon *et al.*¹ were taken as the observed relative risks. The remaining parameter, f_2 , was then fitted by fixing f_1 and b at the values calculated above and comparing the observed TNCS rates over the age range 25–69 yr with the corresponding predicted rates. After completion of this step, the fit of the observed and predicted relative risks for age at FFTP was reassessed and the process continued until stable parameter values were obtained. Throughout the fitting procedure, it was assumed that k was 4.5 and the latent period was 1 yr. The fitting procedure yielded $\hat{f}_1 = 0.70$, $\hat{b} = 2.2$, $\hat{f}_2 = 0.105$ and $\hat{a} = 0.0000515$.

Table 1 Observed and predicted effects of age at menarche, first full-term pregnancy (FFTP) and menopause on breast cancer risk

Risk factor	Age	Observed relative risk	Predicted relative risk
Menarche*	–11	1.00	1.00
	12	0.90	0.77
	13+	0.50	0.59
FFTP†	–19	0.83	0.78
	20–24	1.00	1.00
	25–29	1.30	1.27
	30–34	1.57	1.58
	35+	2.03	1.96
	Nullipars	1.67	1.63
	40–44	1.00	1.00
Menopause‡	45–49	1.27	1.27
	50–54	1.47	1.60
	55–59	2.03	2.00

* From Pike *et al.*⁵. Menarche at 11 or younger set at 11, menarche at 13 or older set at 13, FFTP set at 22, age set at 30.

† From MacMahon *et al.*¹. Menarche set at 13, FFTP less than 20 assumed to be uniform over 15–19, FFTP greater than 35 assumed to be uniform over 35–39, menopause set at 49, age set at 55.

‡ Derived from Trichopoulos *et al.*³. Menarche set at 13, FFTP set at 22, age set at 65.

Table 2 Predicted effects of age at FFTP on breast cancer risk at various ages*

Age at FFTP	Predicted relative risk at age						
	20–24	25–29	30–34	35–39	40–49	50–59	60–69
15–19	1.00	1.00	1.00	1.00	1.00	1.00	1.00
20–24	—	1.65	1.49	1.39	1.31	1.28	1.27
25–29	—	—	2.15	1.89	1.69	1.62	1.60
30–34	—	—	—	2.53	2.15	2.03	1.98
35–39	—	—	—	—	2.70	2.51	2.43
Nulliparous	0.66	1.23	1.72	2.11	2.28	2.09	2.03

* Menarche set at 13, menopause set at 49.

The fitted model

The fit of the model to the TNCS data is shown in Fig. 1. Table 1 shows the observed and predicted relative risks for menarche, FFTP and menopause. The predictions are in excellent agreement with observed values, and we note in particular that the predicted risk for nulliparous women is lower than that of women with a FFTP after age 35.

The predicted effect of age at menarche is most marked at young ages of diagnosis (results not shown): the effect at age 60 is only about 65% of that at age 30. Because errors in recall of age at menarche will also increase with age, this probably explains why certain studies of older breast cancer cases and controls have not found a significant effect of age at menarche.

Table 2 gives the predicted relative risks for age at FFTP by age at diagnosis. Before age 25, the model predicts that nulliparous women will have a lower risk than parous women. This gradually changes with increasing age but even at age 30–34 the model predicts that nulliparous women will have a lower risk than women with FFTP after age 25. Nulliparous women will have a lower risk than women with FFTP after age 35 at all ages.

Discussion

It is difficult to know the relative weights to give to the different sets of information on breast cancer in fitting the model parameters. We make no claim of optimality for our method, but other fitting methods would have to produce estimates that cannot be far different from the values we found if they are to result in an adequate description of all the results. For simplicity we considered breast tissue ageing as starting at the first menstrual period and found a latent period of 1 yr to give the best overall fit. We could almost equivalently have set the latent

Table 3 Mean age at menarche, median age at first birth of parous women, % nulliparous women and adult weight in Japanese and in US whites⁴¹

Birth cohort	Menarche*		First birth†		Nulliparity‡		Weight§	
	Japan	US	Japan	US	Japan	US	Japan	US
1900-04	16.4	14.0	22.7	22.4	12.7	19.8	46.9	68.4
1905-09	16.0	13.6	22.8	22.7	10.9	20.8	49.1	67.3
1910-14	15.7	13.6	23.7	23.6	9.7	18.7	50.1	66.4
1915-19	15.6	13.7	24.4	23.8	8.3	14.4	51.0	65.7
1920-24	15.3	13.4	24.4	23.3	6.5	9.9	51.8	64.4
1925-29	15.2	13.3	23.9	22.4	5.5	9.4	51.7	62.6
1930-34	15.5	13.2	—	—	—	—	50.8	60.9
1935-39	14.9	13.2	—	—	—	—	50.6	59.3
1940-44	14.4	13.1	—	—	—	—	49.5	57.7

* Average age at menarche in years.

† Median age at first birth in years among ever-parous women. Incomplete data after 1930.

‡ Percentage of nulliparous women. Incomplete data from 1930.

§ Average weight in kg. Data for Japanese are from 1970; data for US whites are for 1960-62.

Table 4 Observed and predicted cross-sectional breast cancer incidence rates for Japanese women in 1970

Age	Observed rate*	Predicted rates†		US rate‡
		Model 1	Model 2	
40-44	33.4	79.9	46.1	103.7
45-49	43.4	113.6	64.6	159.2
50-54	41.7	124.1	70.4	171.7
55-59	32.2	121.7	68.8	191.8
60-64	42.8	110.8	62.4	226.2
65-69	35.3	100.0	56.3	234.2

* Per 100,000 (ref. 40).

† From proposed model with appropriate distributions of age at menarche, first birth and menopause from Hoel *et al.*³⁵. In model 1 $f_0 = 1$, $f_1 = f_1$ and $f_2 = 0$. In model 2 $f_0 = 1 - f_2$, $f_1 = f_1 - f_2$ and $f_2 = 0$ (see text).‡ Third National Cancer Survey rates for US whites¹³.

period at 2 yr, for example, and considered breast tissue ageing as starting a year before the first menstrual period. Latent period is an especially complicated concept in breast cancer because its length is also likely to be age dependent—a proportion of breast cancers are still hormone dependent at diagnosis. A more complete model will need to take this into account.

The proposed model provides an excellent shorthand description of several key epidemiological facts concerning breast cancer, and brings the age-incidence curve of this cancer into line with that of the major non-hormone-dependent cancers. The model emphasizes breast tissue age as fundamental to the assessment of breast cancer risk.

The large effect of age at menarche and menopause suggests that hormones have a major role in determining breast tissue age, and oestrogens and prolactin seem to be the major hormones involved. Oestrogens and prolactin increase around menarche and start to decrease in the perimenopausal period²³, in agreement with the start of breast tissue age around menarche and the decreasing rate of breast tissue ageing after age 40. Recent evidence of a decrease in prolactin levels after FFTP²⁴⁻²⁶ suggests that hormonal events may also be responsible for the long-term beneficial effect of FFTP.

The concept of breast tissue age is strongly associated with the cell kinetics of the relevant breast tissue 'stem' cells²⁷. Although the kinetics of the stem cells are not measurable because we cannot specifically identify these cells, one presumes that their kinetics will be closely related to the kinetics of breast epithelial cells as a whole, and the mitotic activity of breast epithelium appears to vary, at least in qualitative terms, in parallel with the rate of breast tissue ageing shown in Fig. 2. There is considerably lower mitotic activity of the breast epithelium after menopause²⁸, and the preliminary kinetic studies

of Anderson and Ferguson²⁹ found the peak mitotic activity in parous women to be ~75% that in nulliparous women, although the results were not statistically significant, possibly due to the extreme person to person variation noted with the single biopsy method they use.

The mitotic activity of breast epithelium varies markedly during the normal menstrual cycle, with the peak activity occurring late in the luteal phase²⁹⁻³², suggesting that, in addition to oestrogen and prolactin, progesterone may be important in inducing mitotic activity in breast epithelium. This effect of progesterone would be in sharp distinction to its effect on endometrial tissue, where the peak mitotic activity is in the follicular phase of the cycle, and suggests that progesterone is part of the biological basis for the epidemiological observation that women who begin cycling regularly within 1 yr of menarche, with associated ovulation and raised progesterone levels, may experience almost twice the breast cancer risk of women who take more than 5 yr to do so³³. Considerably more work is needed to clarify the relationship of breast tissue mitotic activity to parity and hormonal levels, and hence to the model described here.

The model incorporates a gradual slowing down of the rate of breast tissue ageing during the perimenopausal period. We did not need to make such second-order adjustments to describe the effects of age at menarche. However, we noted above that early regular cycling has been found to be associated with increased breast cancer risk³³, and this fact would need to be incorporated into a fully descriptive model.

The model predicts that at young ages nulliparous women will have lower breast cancer rates than parous women (Table 2). Breast cancer rates of married women are much greater³⁴ than those of single women before age 25 and exceed³⁵ the rates of married women only after age 35. This strongly supports the prediction.

The breast cancer risk associated with postmenopausal weight is thought to operate through the associated increased levels of 'free' and total oestrogens derived by peripheral conversion of adrenal androgens³⁶. Such conversion also occurs at premenopausal ages³⁷, and the overall level of oestrogens during a menstrual cycle will thus be positively correlated with weight. This suggests that premenopausal weight should also be slightly positively associated with breast cancer risk. The data of de Waard *et al.*⁷ and of Lew and Garfinkel⁸ are inadequate to investigate this issue properly.

There is remarkable international variation in breast cancer rates^{13,38,39}. As of 1970, age-adjusted rates were some five to six times higher in the United States than in Japan. The differences in rates do not seem to be due to differences in genetic susceptibility⁴⁰, and it is therefore instructive to calculate the breast cancer rates that the model would predict for Japan.

The necessary data on menarche, first birth, menopause and weight, to predict the Japanese rates between ages 40 and 69, are available from a 1970 survey in Hiroshima and Nagasaki⁴¹. Some of the results are given in Table 3, together with the comparable figures for US whites. The average age at natural menopause of Japanese women was comparable with that of American women, and fewer Japanese had surgical menopause. The data on menarche and weight favour a lower breast cancer rate in Japanese women. Older Japanese women had a much later menarche, and weighed 13-21 kg less than American women. The data on first birth, nulliparity and menopause show that none of their decreased breast cancer rate can be attributed to these factors.

Japanese breast cancer rates remain almost constant after age 50⁴². In model terms this implies that for Japanese women, we must set f_2 (Fig. 2) to zero. This is, in all probability, a weight effect; in 1970 the average weight of postmenopausal Japanese women was less than 50 kg, and these women are unlikely to have been producing significant amounts of oestrogens. Their breast tissue age will advance little, if at all, and their breast cancer incidence will remain almost constant, as is observed.

The rates predicted for the Japanese by the model with the assumption of $f_0 = 1$, $f_1 = \hat{f}_1$ and $f_2 = 0$ are given in Table 4 as model 1. Although these predicted rates are considerably lower than the observed rates of US whites, they are still much greater than the observed Japanese rates.

As discussed above, peripheral conversion of androgens to oestrogens also takes place in the premenopausal period, and this suggests that we should reduce f_0 and f_1 from $f_0 = 1$ and f_1 to $(1 - \hat{f}_2) = 0.895$ and $(\hat{f}_1 - \hat{f}_2) = 0.595$ to model this postulated premenopausal weight effect; this greatly improves the fit of the model (model 2 in Table 4). The predicted rates are still, however, some 1.6-fold greater than the observed rates and suggests that it may be useful to look for an additional Japanese factor. Analysis shows that the 1.6-fold difference between the observed and predicted rates may be accounted for by slowing down breast tissue ageing by only 11%. Most studies are not designed to find such small differences statistically significant.

The remaining unexplained differences between the two countries' breast cancer rates may be dietary fat⁴³. However, in the United States the later menarche occurs the longer it takes to establish regular cycles¹⁵, and there may therefore be a greater delay in the establishment of regular cycles in Japanese women. Such a delay would further decrease their breast cancer risk³³ and could explain much of the remaining unexplained differences.

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Escherichia coli single-strand binding protein stabilizes specific denatured sites in superhelical DNA

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Escherichia coli single-strand binding protein relaxes supercoiled DNA molecules containing the *Drosophila melanogaster* histone gene repeat unit, by stabilizing denaturation bubbles that map near the boundaries of the genes, at sites that in native chromatin have been shown to be hypersensitive to nucleases. A similar process may contribute to the propagation of such hypersensitive sites after their induction on the activation of gene expression.

ACTIVE chromatin displays discrete exposed DNA regions near the 5', and sometimes also near the 3', boundaries of genes^{1–5}. These sites are hypersensitive to DNase I when the genes are

active, but they show no preferential sensitivity to the nuclease when the genes are inactive^{2,6}. The DNase I hypersensitive sites are among the most intriguing and characteristic features of

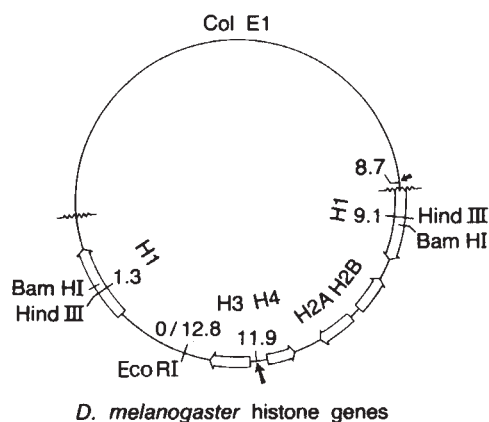


Fig. 1 Map of the plasmid cDm506. Open arrows show the direction and extent of transcription of the *D. melanogaster* histone genes^{15,16}. Numbers indicate the distance, in kb, from the single *EcoRI* site. The large and the small arrow point to the position of, respectively, the major and minor S₁ sensitive sites in the supercoiled DNA molecule.

active eukaryotic genes. It is becoming increasingly clear that the DNA sequences at those sites are peculiar in that they appear to be particularly susceptible to nucleases and chemical cleavage in protein-free DNA^{7,8}. Furthermore, the sites are sensitive to single-strand specific nucleases in chromatin, and also in the deproteinized supercoiled DNA⁹, which suggests that they may contain hairpin structures of the type recently described¹⁰⁻¹⁴. Groudine and Weintraub have recently shown that, once induced, the DNase I hypersensitive sites are inherited in the absence of the original inducer¹⁴. They have also suggested a simple model by which DNA single-strand binding (s.s.b.) proteins could stabilize and propagate such hypersensitive sites during chromatin replication¹⁴.

Recent studies in our laboratory have focused on the *Drosophila melanogaster* histone genes, which are particularly suited for structural analysis. The five histone genes are clustered in a 5,000-base pair (bp) repeat unit¹⁵ whose nucleotide sequence has been almost completely determined¹⁶. The chromatin structure of the repeat unit has also been examined, and the DNase I and micrococcal nuclease hypersensitive sites have been mapped to the 5' and 3' boundaries of the five histone genes⁴. We decided to test whether the DNA sequence of the histone gene repeat contains structural information which could be used to build the specific chromatin structures observed. In particular, we wished to determine and map the sites detected by single-strand specific nucleases and by s.s.b. proteins in the supercoiled DNA. As we report here, the *E. coli* s.s.b. protein melts the supercoiled DNA at specific sites. The discrete denaturation sites map to the 5' and 3' boundaries of the genes, at, or very near, the sites detected by DNase I and micrococcal nuclease in chromatin.

Mapping the S₁ nuclease-sensitive sites

The plasmid cDm506 contains an entire *D. melanogaster* histone gene repeat unit, together with a bit over including a second copy of H1 gene (Fig. 1). To locate the transient single-stranded regions present in the supercoiled (form I) DNA^{12,13}, cDm506 was digested with the single-strand specific nuclease S₁, and the S₁ cutting sites were mapped by further cleavage with *Hind*III and/or *Eco*RI. As shown in Fig. 2, the plasmid preparation we used contained some relaxed DNA (lane 1, form II DNA). S₁ nuclease treatment linearized the supercoiled DNA to form molecules that migrate slightly above the form I DNA (lane 2, form III DNA), whilst the relaxed form was apparently not cleaved by S₁ nuclease. Digestion of the S₁-cleaved DNA with *Hind*III produced two new bands of 2.8 and 2.2 kilobases (kb), derived from the 5.0 kb histone gene

repeat unit (compare lanes 6 and 3), whilst *Eco*RI generated a 11.9 and 0.9 kb fragment (compare lanes 7 and 4). Double-digestion of the cDm506 plasmid with *Hind*III and *Eco*RI generated fragments of 7.8, 3.7 and 1.3 kb (Fig. 2, lane 8), while similar double digestions performed after S₁ treatment (Fig. 2, lane 14) generated in addition, 2.8 and 0.9 kb DNA fragments derived from the 3.7 kb *Hind*III-*Eco*RI restriction fragment.

As shown by the large black arrow in Fig. 1, the S₁-generated fragments unambiguously locate the major S₁-cleavage site in the small intergenic spacer separating the 5' ends of the divergent H3 and H4 histone genes. A (GA)₁₀ sequence present in this spacer¹⁶ may be the actual site of S₁ cleavage, by analogy with the S₁-sensitivity of a similar sequence, (GA)₁₆, in the sea urchin histone repeat unit¹⁷. A number of minor S₁-cleavage sites are also found, one, for example, mapping near the former *Eco*RI site in ColE1 (see small black arrow in Fig. 1) and which probably corresponds to the S₁ site mapped by Lilley¹² at that same location in the supercoiled ColE1 DNA.

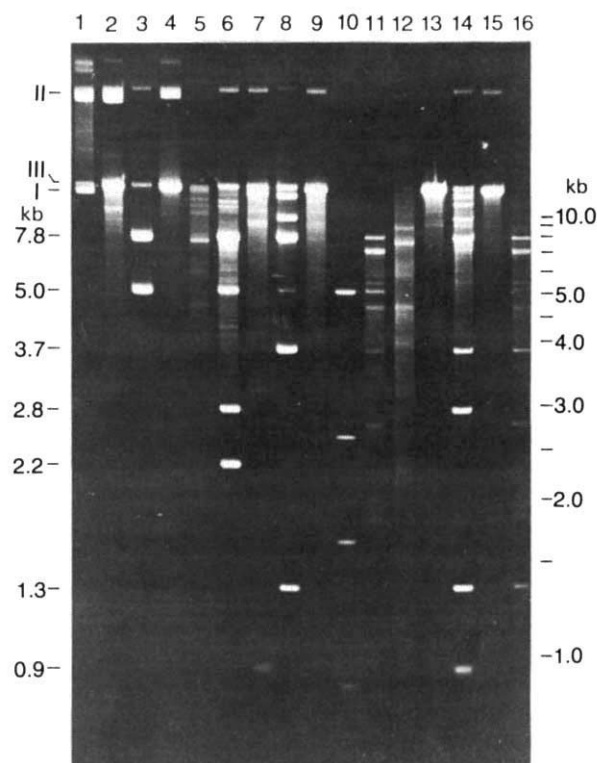


Fig. 2 Mapping of the S₁-cleavage sites. The lanes were loaded with cDm506 plasmid that had been treated with the following enzymes: 1, none; 2, S₁; 3, *Hind*III; 4, *Eco*RI; 5, S₁ after preincubation with *E. coli* s.s.b. protein; 6, *Hind*III after S₁; 7, *Eco*RI after S₁; 8, *Hind*III and *Eco*RI; 9, S₁ after *Eco*RI; 10, DNA molecular weight markers; 11, s.s.b. protein followed by S₁ and then *Hind*III; 12, s.s.b. protein followed by S₁ and then *Eco*RI; 13, s.s.b. protein followed by *Eco*RI and then S₁; 14, *Hind*III and *Eco*RI after S₁; 15, *Eco*RI followed by s.s.b. protein and then S₁; 16, s.s.b. protein followed by S₁ and then *Hind*III and *Eco*RI.

Methods: Purified plasmid DNA (1 µg) was preincubated 10 min at 37 °C, with or without 0.37 µg of purified *E. coli* s.s.b. protein, in 10 µl of buffer (10 mM Tris-HCl pH 7.4, 50 mM NaCl, 1 mM EDTA). This preincubation was followed by addition of 10 µl of H₂O, 20 µl of 2X S₁ buffer¹² and 1 unit of S₁ endonuclease³². S₁ digestion proceeded for 15 min at 37 °C. The reaction was terminated by phenol, the DNA was purified by phenol and chloroform extractions, precipitated with ethanol and resuspended in suitable buffers for the various restriction enzymes. In the experiment shown in lane 13, *Eco*RI digestion was performed immediately after preincubation with s.s.b. protein, and this was followed 60 min later by S₁. The DNA samples were electrophoresed in 1% agarose gels, followed by ethidium bromide staining and photography under a UV light source.

Unlike the major S_1 -sensitive site in the *D. melanogaster* histone gene unit, cleavage by S_1 at the (GA)₁₆ site in the sea urchin histone DNA does not require DNA supercoiling and readily occurs in a linearized restriction fragment¹⁷. Probably the smaller (GA) stretch in *D. melanogaster* (10 instead of the 16 present in the sea urchin) requires a higher free energy to flip into a stable, out-of-register, single-strand looped DNA molecule¹⁷.

S_1 -cleavage of DNA-s.s.b. protein complex

Preincubation of the DNA with the *E. coli* s.s.b. protein¹⁸ drastically changes the pattern of S_1 cleavage (Fig. 2). Many new discrete sites appear which are cleaved by S_1 , while the original site at the H3-H4 intergenic spacer is less frequently cleaved. Controls without S_1 showed that the s.s.b. protein by itself, purified as described by Chase *et al.*¹⁹, has no nuclease activity. The new S_1 -generated DNA fragments are displayed in Fig. 2 after *Hind*III digestion (compare lanes 11 and 6), and after *Eco*RI digestion (compare lanes 12 and 7). Note that there is an absolute requirement of supercoiled DNA for s.s.b. protein binding and S_1 cleavage. Treatment of the supercoiled DNA with *Eco*RI, either before or, what is even more significant, after binding the s.s.b. protein, erases all of the S_1 sensitive sites (compare lane 12 with lanes 13 and 15).

It is rather difficult to map the s.s.b. protein-induced S_1 -sensitive sites displayed in Fig. 2 because of their large number, and also because each of the S_1 cuts generates two DNA fragments on further digestion with restriction enzymes. To locate the sites denatured by the s.s.b. protein in the *D. melanogaster* histone genes we used a different approach, as described below.

Mapping the s.s.b. protein-induced S_1 sites

We have developed a procedure for internally 'tagging' a supercoiled DNA molecule at a single restriction site²⁰. A restriction fragment, in this case the *Hind*III (or *Bam*HI) 5,000 bp histone gene unit shown in Fig. 1, is labelled at the 5' ends by treatment with polynucleotide kinase and ³²P-ATP²¹ and ligated. The two ³²P labels remain in the reformed restriction site of the closed-circular DNA molecule, serving as a reference point for future analysis. The labelled closed-circular DNA is then made superhelical by injection into the *Xenopus* oocyte nucleus²².

Lane 2 in Fig. 3 shows the electrophoretic mobility of the *Hind*III labelled DNA substrate extracted from *Xenopus* oocytes. About two-thirds of the molecules are supercoiled (form I DNA). Digestion with *Bam*HI (lane 3) generates an 'aligned' 5,000 bp linear DNA molecule carrying the two ³²P labels at the *Hind*III site, 68 bp away from one of the *Bam*HI ends (see left diagram in Fig. 3). All the S_1 cleavage sites can then be accurately mapped by direct comparison with known internal restriction sites (see lane 1).

As expected, the supercoiled DNA is linearized by S_1 nuclease, while the relaxed form II DNA is uncleaved (compare lanes 4 and 2). Further digestion with *Bam*HI linearizes the form II DNA and, in addition, generates a new labelled *Bam*HI- S_1 fragment derived from the S_1 -linearized DNA molecules (compare lanes 7 and 4). Also as expected, the S_1 cleavage maps near or at the (GA)₁₀ site within the H3-H4 DNA spacer as found previously (see Figs 1 and 2).

Lanes 5 and 6 show the effect of preincubation of the supercoiled DNA with s.s.b. protein followed by S_1 treatment. As before (compare with lane 2), the supercoiled molecules are linearized by S_1 , while the relaxed ones are not cleaved. A small number of molecules are cleaved more than once, generating linear fragments of less than 5,000 bp. Digestion with *Bam*HI generates a discrete set of *Bam*HI- S_1 fragments (lanes 8, 9); the weak bands in the upper region of the gel can be mapped more accurately using the *Bam*HI labelled, *Hind*III-aligned DNA (see lanes, 10, 11, and diagram on the right in Fig. 3). Since most of the s.s.b. protein-DNA complexes are cleaved only once by S_1 (see lanes 5, 6), the various discrete

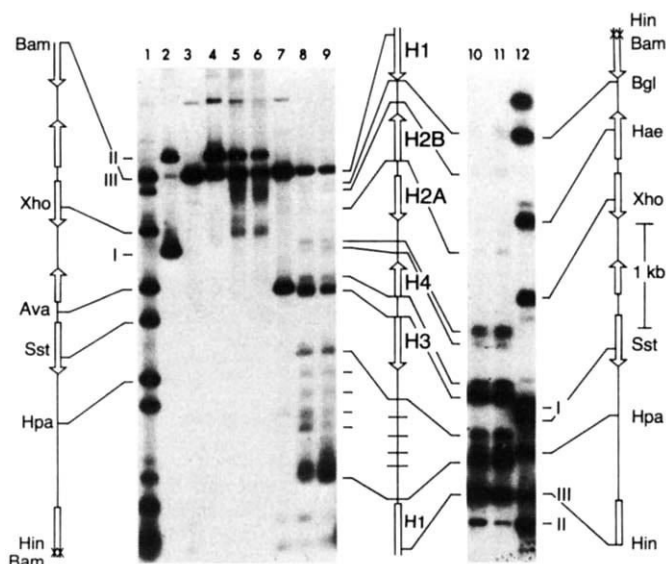


Fig. 3 Mapping of the s.s.b. protein-induced S_1 sites in the supercoiled histone gene DNA. *Hind*III (or *Bam*HI) single-site labelled supercoiled DNA was prepared as described²⁰. Lanes 1-9, *Hind*III labelled DNA; lanes 10-12, *Bam*HI labelled DNA. The lanes were loaded with ³²P labelled DNA that had been treated with the following enzymes: 1, DNA restriction fragments as markers; 2, untreated DNA; 3, *Bam*HI; 4, S_1 ; 5, s.s.b. protein (0.4 μ g) followed by S_1 ; 6, s.s.b. protein (0.8 μ g) followed by S_1 ; 7, *Bam*HI digestion of S_1 -treated DNA; 8, 9, *Bam*HI digestion of s.s.b. protein (0.4 μ g, 0.8 μ g) and S_1 -treated DNA; 10, 11, *Hind*III digestion of s.s.b. protein (0.4 μ g, 0.8 μ g) and S_1 -treated DNA; 12, DNA restriction fragments as markers.

Methods: Preincubations and incubations were performed using 1 μ g of cold carrier cDm506 plasmid as described in the legend to Fig. 2. After 1% agarose gel electrophoresis, the gels were dried and exposed to Kodak X-ray film. The radioautograph shown in lanes 10, 11 and 12 has been inverted (smaller DNA fragments are in the upper part of the picture).

S_1 -cleavages displayed in lanes 8-11 must belong to different DNA molecules, each DNA molecule carrying a single s.s.b. protein-induced S_1 site.

In addition to generating new S_1 -sensitive sites, the s.s.b. protein decreases the frequency of S_1 -cleavage at the (GA)₁₀ site. Instead, two new cleavage sites appear on either side of the (GA)₁₀ site. The two new sites map to the 5' ends of the H3 and H4 genes. The other major s.s.b. protein-induced S_1 site maps to a region near the 5' end of the H1 gene. Another S_1 site is located at the 5' end of the H2B gene, while the remaining sites map within the H1-H3 DNA spacer, the H4-H2A DNA spacer and the H2B-H1 DNA spacer. No S_1 cleavages can be detected within the genes. Note that the s.s.b. protein-induced S_1 sites on the supercoiled DNA appear to be the same ones (± 20 bp) as the sites exposed and preferentially cleaved in chromatin by mild micrococcal nuclease and DNase I digestions^{4,23}.

Electron microscopic visualization

To gain an insight into the DNA structural change brought about by the *E. coli* s.s.b. protein, we examined the plasmid cDm506 DNA with the electron microscope after glutaraldehyde fixation, before and after binding to the protein. As can be seen by comparing the upper left and right panels in Fig. 4, the s.s.b. protein-DNA complexes look less supercoiled than the protein-free supercoiled DNA, and they all display a single denaturation bubble equal in length to $2.7 \pm 0.04\%$ of the total DNA length (average of 70 measurements).

The position of the denaturation bubble can be mapped by digestion with *Eco*RI (lower left panel). Of 50 linear, *Eco*RI-cleaved DNA molecules traced, 30 carried the bubble at a

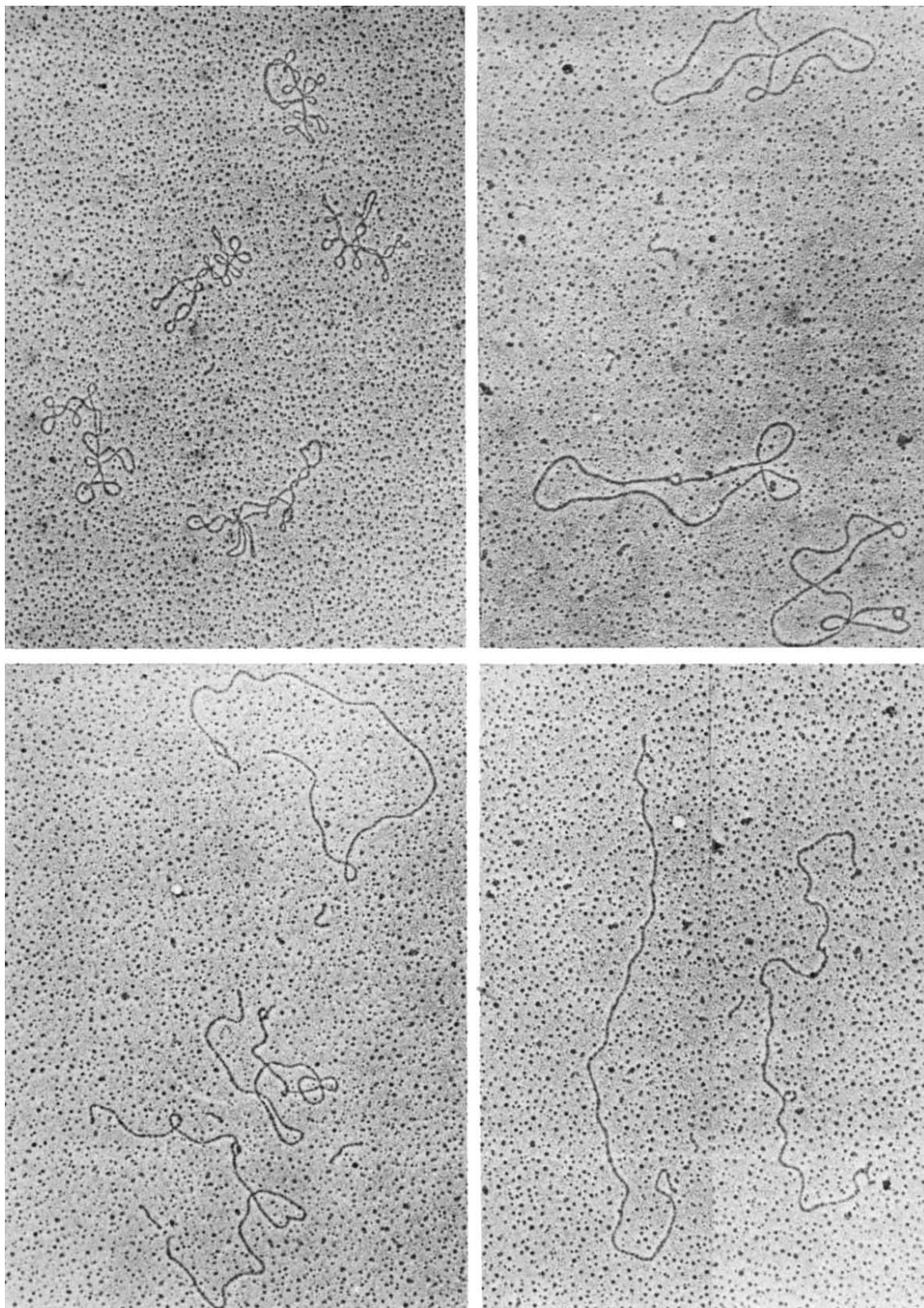


Fig. 4 Electron micrographs of the cDm506 plasmid. Upper left, protein-free supercoiled DNA; upper right, s.s.b. protein-DNA complexes; lower left, s.s.b. protein-DNA complexes after *EcoRI* cleavage; lower right, s.s.b. protein-DNA complexes after *S*₁ cleavage.

Methods: 1 μ g of DNA was preincubated at 37 °C for 10 min in the absence or presence of 0.6 μ g of *E. coli* s.s.b. protein in 16 μ l of 0.01 M sodium phosphate buffer (pH 6.8), then glutaraldehyde was added to a final concentration of 10 mM and the mixture was kept for another 10 min at the same temperature. Fixation was stopped by addition of 1 μ l of 1.0 M Tris-HCl (pH 7.9), the samples were diluted to 60 μ l with H₂O, and spread by the formamide spreading technique³³, before or after treatment with *EcoRI* or *S*₁. *EcoRI* or *S*₁ digestions were performed as described in the legend to Fig. 2, except that 10 times higher *S*₁ concentrations were used. All the photographs ($\times 36,000$), were taken with a Zeiss EM 109. The size and position of the denatured region were determined by tracing the molecules with a Tektronic digitizer.

position about 4,000 bp $\pm$ 700 bp away from one end, like the molecule shown in the lower part of the panel. This major s.s.b. protein site may well be the same site previously mapped in ColE1 at 8.7 kb from the *EcoRI* site (see Fig. 1). The remaining 20 molecules carried the denaturation bubble at various positions near one *EcoRI* end, like the two molecules shown in the upper part of the panel. Such molecules must be plasmids with a melted site within the *D. melanogaster* DNA; the rather large error inherent in the electron microscopic measurements precluded precise mapping.

The lower right panel of Fig. 4 shows two linear molecules produced by S_1 cleavage of the s.s.b. protein-DNA complex. While the glutaraldehyde-fixed complexes were readily cleaved by *EcoRI*, they were resistant to S_1 nuclease, presumably because of steric hindrance of the cross-linked s.s.b. protein. Thus, fewer linear DNA molecules were produced, even at high S_1 nuclease concentrations. However, most of the S_1 -generated linear molecules examined had whiskers at one end, and none showed a denaturation bubble inside the linear molecule of the type seen in the *EcoRI*-cleaved complexes. We might thus conclude that S_1 cleaves at the site of the denaturation bubble, and that there probably is a one-to-one correlation between the denaturation bubble seen with the electron microscope and the s.s.b. protein-induced sites mapped by S_1 cleavage in Figs 2 and 3.

Discussion

Thermal fluctuations in the supercoiled DNA will generate transient denaturation bubbles which are recognized and cleaved by S_1 (refs 12, 13). At physiological ionic strength and temperature, the equilibrium is very much displaced towards the native Watson-Crick DNA form, but, given the irreversible nature of the S_1 cleavage, eventually all supercoiled molecules will be linearized through such a pathway. In the presence of *E. coli* s.s.b. protein, on the other hand, supercoiled DNA molecules will tend to adopt a partially melted conformation. Electron microscopic examination of the s.s.b. protein-DNA complexes reveals that none of the DNA molecules appears fully native and supertwisted, but instead, they display a discrete denaturation bubble. It is therefore likely that in the presence of the s.s.b. protein the equilibrium is shifted towards the partially melted supercoils.

The s.s.b. protein driven denatured state must be highly metastable, since restriction cleavage results in fully native

linear DNA molecules (compare lanes 12 and 13 in Fig. 2). Similarly, treatment with nicking-closing enzyme fully relaxes the s.s.b. protein-DNA complexes (G.C.G., unpublished observations). Thus, the denaturation bubble is locked-in only as long as the DNA is fully supercoiled.

The *E. coli* s.s.b. protein can recognize and melt sites that are different from the major S_1 -sensitive site. This effect may be due to the cooperative nature of binding of the s.s.b. protein^{18,24}. While S_1 probably requires stable DNA single-stranded structures for efficient binding and cleavage, a transitory melting of just eight base pairs will allow binding of the s.s.b. protein²⁴, which will then facilitate the binding of neighbouring protein subunits. This zipper-like action will continue until most of the superhelices are dissipated into the denaturation bubble.

What could be the biological relevance of such localized denaturation bubbles? Since the finding that the *E. coli* chromosome is supercoiled²⁵, it has been generally assumed that the DNA is actually supertwisted inside the cell. The fact that *E. coli* s.s.b. protein, in physiological amounts and physiological conditions, stabilizes partially melted supercoils *in vitro* implies that the same may also hold *in vivo*. We therefore suggest that chromosomal DNA in *E. coli* may actually contain such metastable denaturation bubbles. As has been stressed by several workers, localized denatured regions in DNA must have an important role in initiation of DNA replication^{26,27}, transcription²⁸ recombination^{29,30} and translocation³¹.

Analysis of the DNA sequence of the *D. melanogaster* histone repeat¹⁶ reveals that, as might have been expected, the sites melted by the *E. coli* s.s.b. protein (Fig. 3) are relatively A + T rich. The same sites are also recognized and preferentially cleaved by micrococcal nuclease and *o*-phenanthroline in DNA⁷, and by micrococcal nuclease and DNase I in chromatin^{4,23}. In spite of the fact that the DNase I hypersensitive sites in chromatin and the s.s.b. protein-induced single-strand DNA regions in supercoiled DNA map at similar sites in the *D. melanogaster* histone gene unit, it remains to be established whether, as suggested¹⁴, s.s.b. proteins have a role in the propagation of the nuclease-hypersensitive sites during chromatin replication.

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Acquisition of transforming properties by alternative point mutations within *c-bas/has* human proto-oncogene

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The transforming gene of a human lung carcinoma-derived cell line, Hs242, has been cloned in biologically active form, and identified as c-bas/has (otherwise known as c-Ha-ras). The genetic lesion responsible for the transforming activity of the Hs242 oncogene has been localized to a point mutation in the second exon which results in the substitution of leucine for glutamine as amino acid 61 of the predicted protein. No changes were observed in the first exon, the region of c-bas/has in which a point mutation is responsible for activation of the T24 and EJ bladder carcinoma oncogenes.

INVESTIGATIONS of the genetic alterations that cause normal cells to become malignant have recently focused on a small set of cellular genes. Acute transforming retroviruses have substituted viral genes necessary for replication with these discrete segments of host genetic information¹. When incorporated within the retroviral genome, such transduced sequences derived from normal cellular genes (proto-oncogenes) acquire the ability to induce neoplastic transformation. Findings that independent virus isolates have often recombined with the same or closely related proto-oncogenes²⁻⁴ have implied that only a limited number of cellular genes are capable of acquiring transforming properties in these conditions.

The development of DNA-mediated gene transfer techniques has provided another approach for the detection of cellular transforming DNA sequences. DNAs from human tumours and tumour cell lines have been found to induce malignant transformation of NIH 3T3 cells⁵⁻¹², a continuous murine cell line which is contact-inhibited and highly susceptible to DNA transfection. A large proportion of such transforming genes so far detected are related to a small family of retroviral *onc* genes. The oncogenes, *bas* and *has*, of BALB- (ref. 13) and Harvey(Ha)-murine sarcoma viruses (MSV)¹⁴ derive from the same proto-oncogene, independently transduced from mouse and rat species, respectively^{4,15}. The Kirsten(Ki)-MSV¹⁶ *onc* gene, *kis*, originates from a different rat proto-oncogene, but is related to *bas/has* at the nucleotide sequence and protein coding levels (refs 17-19 and E.P.R. *et al.*, in preparation). Dominant transforming genes detected in diverse human tumours including carcinomas of the digestive tract, lung and urinary bladder as well as a rhabdomyosarcoma, are related to *kis*^{12,20}. Moreover, transforming genes isolated by molecular cloning techniques from human bladder carcinoma lines, EJ and T24, represent activated *bas/has* (human) proto-oncogenes²⁰⁻²².

During the analysis of human cells for transforming DNA sequences, we detected a transforming gene related to *c-bas/has* (human) in a cell culture derived from a human lung carcinoma, Hs242. This allowed us to molecularly clone and compare the mechanisms by which the same human proto-oncogene has been independently activated in human tumour-derived cells.

Identification of Hs242 oncogene

Hs242 cells were obtained at sixth passage from the Naval Biosciences Laboratory, Oakland, California. The cells had been initiated as an explant of a lung carcinoma of a 63-yr-old caucasian female. When high-molecular weight DNA of Hs242 cells was subjected to transfection analysis using NIH 3T3 cells, we observed a low level of focus formation with independently prepared DNA preparations (Table 1). The specific activity of

the transforming DNA was increased 10-25-fold by a second cycle of transfection using high-molecular weight DNAs of individual first-cycle NIH 3T3 transformants. Transforming activity also survived a third cycle of transfection (Table 1).

To confirm that the foci observed were induced by human DNA, the DNAs of individual transfectants were subjected to Southern blotting analysis using a probe specific for human 'Alu' repetitive sequences (ref. 23). Each first-cycle transfectant tested demonstrated numerous bands containing human repetitive sequences (see Fig. 1A, lane 1). Third-cycle transfectants digested with *EcoRI* invariably retained an *Alu*-containing band of >20 kilobase pairs (kbp) (Fig. 1A, lanes 2, 3), implying that human sequences were linked to the transforming sequence.

In view of recent evidence relating transforming DNA sequences of a number of human tumours to *v-kis* or *v-bas*^{12,20-22}, we analysed Hs242 transfectant DNAs for sequences related to these transforming genes. The *v-kis* probe did not detect any bands other than the endogenous *v-kis*-related bands (data not shown). However, when *BamHI*-digested Hs242 transfectant DNAs were analysed using *v-bas* as a probe, each exhibited a 7-kbp fragment in addition to the endogenous 3.5-kbp band (Fig. 1B). The extra *v-bas*-related DNA fragment persisted in all second- and third-cycle transfectants tested, implying that a human gene related to *v-bas* co-segregated with the observed transforming activity. These results were further confirmed by using *c-bas/has* (human) as a probe.

A representative third-cycle Hs242 transfectant demonstrated a single DNA fragment of molecular weight (MW)

Table 1 Transforming activity of DNA isolated from Hs242 cells and their NIH 3T3 transfectants

Donor DNA	Transfection efficiency (no. foci/no. recipient cultures)		
	Primary	2nd cycle	3rd cycle
Hs242			
Preparation 1	2/9	17/8	36/8
Preparation 2	2/14	29/8	30/8

Two independently prepared DNAs from human lung carcinoma-derived Hs242 cells as well as representative first- and second-cycle NIH 3T3 transfectants were used to transfect NIH 3T3 cells by the calcium phosphate precipitation method³⁵, as described previously^{10,36}. NIH 3T3 cells, plated 24 h earlier at 1.3×10^5 cells per 10-cm Petri dish, were transfected with 30 µg of high-molecular weight DNA. Cells were maintained in culture with twice weekly changes of Dulbecco's modified Eagle's medium supplemented with 5% calf serum. Foci of transformed cells were scored at 14-21 days.

~7.0 kbp following *Bam*HI digestion and Southern blotting and hybridization with nick-translated *c-bas/has*(human) DNA (Fig. 1C, lane 2). In these conditions, the endogenous *c-bas/has* mouse gene was not detectable (Fig. 1C, lane 1), supporting the human origin of the 7-kbp fragment in the Hs242 transfectant. Blotting analysis of parental Hs242 cell DNA subjected to *Bam*HI digestion revealed two *c-bas/has*(human) alleles of 7 and 8 kbp (Fig. 1C, lane 3). The size of the lower molecular weight species was consistent with that of the *c-bas/has* allele, which segregated with the transforming activity of Hs242 cellular DNA.

Molecular cloning

To characterize the *c-bas/has*(human)-related transforming gene associated with Hs242 cells, we decided to clone this sequence from a second-cycle transfectant induced with *Bam*HI-cleaved 45-3 (the first cycle transfectant) cellular DNA. This transfectant yielded a single *c-bas/has*(human)-related *Eco*RI DNA fragment of 6.0 kbp suitable for cloning in phage λ gtWES- λ B. Following partial purification of ~20-fold by electrophoresis in a low melting point 1.0% agarose gel, DNA of apparent MW 6–7 kbp was ligated to phage DNA, packaged and used to infect *Escherichia coli*. From about 30,000 plaques screened, two positive clones were obtained.

Following plaque purification, the clones were subjected to agarose gel electrophoresis and blotting analysis after *Eco*RI cleavage. Each insert demonstrated the expected size of 6.0 kbp (data not shown). We also prepared subclones of one of the λ clones (λ Hs-2) using a pBR322 vector. The cloned DNAs were next analysed for transforming activity in the NIH 3T3 transfectant assay. As shown in Table 2, the pBR subclones pHs-49 and pHs-125 demonstrated high titres of focus-forming activity. The purified insert from λ Hs-2 had a specific transforming activity of $>10^4$ focus-forming units (FFU) per μ g, comparable with that of the most potent retroviral *onc* genes.

Characterization

The Hs242 transforming sequence was subjected to restriction enzyme analysis in order to compare its physical map with that of the previously reported T24 and EJ bladder tumour oncogenes^{22,24,25} as well as *c-bas/has*(human), cloned from a human fetal liver library²². Figure 2 shows that the restriction map of the Hs242 gene closely corresponded with both oncogenes. Divergence was noted beyond the *Xho*I site at the 5' end and the *Sph*I site at the 3' end of the Hs242 sequence. Presumably this was due to rearrangements introduced at the ends of the DNA during the transfection process. Moreover, this divergence was outside the corresponding region of the T24 oncogene, previously shown to be required for its transforming activity²². By molecular hybridization, its *v-bas*-related sequences were localized to the 2.9-kbp *Sac*I fragment of the Hs242 transforming gene (Fig. 2).

Table 2 Transforming activity of cloned DNA containing Hs242 oncogene

Donor DNA	DNA added (μ g)				Transforming activity (FFU per μ g)
	1.0	0.1	0.01	0.001	
Purified insert from λ Hs-2	TMC	TMC	$>100^*$	30, 30	3×10^4
Plasmid pHs-49	TMC	>100	30, 35	3, 0	3×10^3
pHs-125	TMC	>100	6, 10	0, 1	8×10^2
<i>c-bas/has</i> (human)	0, 0	0, 0			$<10^0$

The indicated amount of donor DNA was mixed with 40 μ g of calf thymus DNA as a carrier and transfected onto NIH 3T3 cells as described in Table 1. TMC, Too many to count.

*Number of foci per plate.

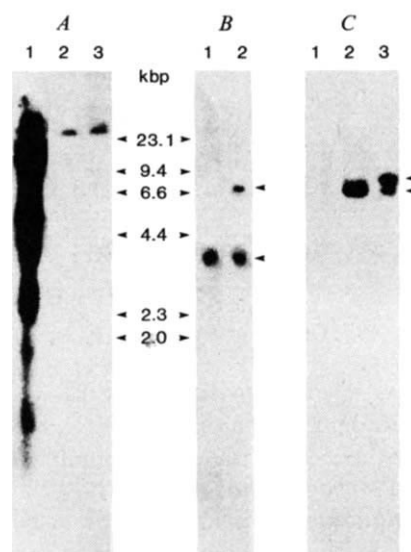


Fig. 1 Detection and identification of the Hs242 transforming gene; 20 μ g of high-molecular weight DNA were digested with 100 U of the appropriate restriction enzyme, electrophoresed in horizontal agarose (1% w/v) gels and blotted to nitrocellulose as described by Southern³⁷. Filters were hybridized in stringent conditions (50% formamide, $5 \times$ SSC, 42 °C) for 24 h. Co-electrophoresed DNA fragments of *Hind*III-digested λ c1857 DNA served as standards (labelled in kbp). Migration of hybridized fragments is indicated by arrowheads (B and C). A, A representative first-cycle 45-3 (lane 1) and two third-cycle transfectant DNAs (lanes 2 and 3) were digested with *Eco*RI and hybridized to 300 bp of human repetitive *Alu* sequences²³. B, NIH 3T3 (lane 1) and 45-3 (lane 2) DNAs were digested with *Bam*HI and hybridized to 675 bp of *v-bas*⁴. C, NIH 3T3 (lane 1), 45-3 (lane 2) and Hs242 cell (lane 3) DNAs were digested with *Bam*HI and hybridized to 6.6-kbp *Bam*HI fragment of *c-bas/has*(human)²².

The translational product of *c-bas/has*(human) has been identified as a protein with a apparent molecular weight of 21,000 (p21), using antisera directed against the BALB- or Ha-MSV transforming gene products²⁶. Previous studies have shown that the T24 and EJ oncogenes, which represent activated *c-bas/has* alleles, code for antigenically related proteins, whose mobility has been altered^{20,22,27,28}. Moreover, the altered mobility of the T24 and EJ products seems to be associated with the alteration in amino acid sequence responsible for acquisition of transforming activity^{27–29}.

To characterize the Hs242 transforming gene product, we used monoclonal antibody prepared against Ha-MSV p21 (ref. 30). As shown in Fig. 3, an Hs242 primary transfectant (lane 2), as well as transformants induced by the cloned Hs242 oncogene (lane 3) demonstrated two p21-related proteins. Notably, these proteins differed in their molecular weights from both species of p21 present in a representative T24 transformant (Fig. 3, lane 4). These results indicated that the genetic alterations responsible for acquisition of transforming activity by the Hs242 oncogene probably differ from those of the T24 oncogene.

Exon localization

Previous studies have demonstrated that the region of the T24 and EJ oncogenes that confers transforming activity on the normal *c-bas/has* allele, encompasses the first exon of its coding sequence^{27–29}. The only sequence difference between the EJ or T24 transforming and nontransforming *c-bas/has*(human) allele has been identified as a single base change of G to T in codon 12 of this coding region. This results in a change of glycine to valine in the predicted amino acid sequence.

The restriction enzyme *Msp*I recognizes CCGG (I. Shildkraut and L. Greenough, unpublished results). A single base change

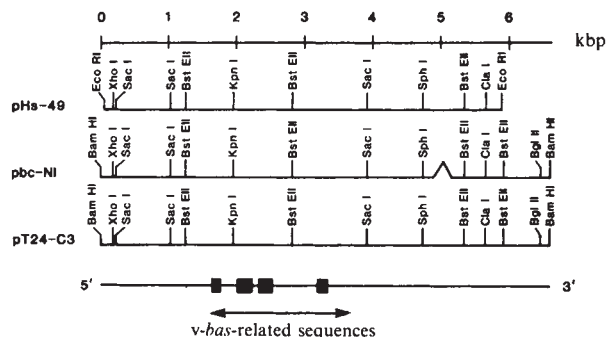


Fig. 2 Comparative endonuclease cleavage maps of the Hs242 oncogene, *c-bas/has* (human) and the T24 oncogene. The diagram depicts the location of the cleavage sites for *Xho*I, *Sac*I, *Bst*EII, *Kpn*I, *Sph*I, *Cla*I and *Bgl*II within subcloned human DNA fragments containing the Hs242 oncogene (pHs-49), *c-bas/has* (human) (pbc-N1) and the T24 oncogene (pT24-C3)²². The restriction maps of *c-bas/has* (human) and the T24 oncogene have been reported elsewhere²². Δ , Indicates deletion in pbc-N1. The locations of the four exons (■) of this gene, as well as its *v-bas*-related sequences, are depicted as previously determined²⁸.

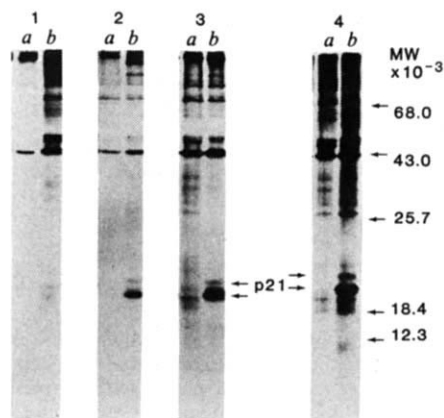


Fig. 3 Detection of proteins antigenically related to the *bas/has* gene products in NIH 3T3 transfectants. Cells were labelled with ³⁵S-methionine at 37°C for 3 h and extracts were prepared and immunoprecipitated as described elsewhere³⁰ using: *a*, normal rat serum or *b*, anti-Ha-MSV p21 monoclonal antibody (A26-YA6-172). Lane 1, uninfected NIH 3T3 cells; 2, a representative Hs242 transfectant; 3, a pHs-49 (the plasmid containing the Hs242 oncogene) transfectant; 4, a T24 transfectant. Molecular weight (MW) markers were co-electrophoresed with the above samples in 12% SDS-polyacrylamide gels. Migration of proteins antigenically related to Ha-MSV p21 is indicated by arrows.

of G to T in codon 12 changes the sequence from glycine to valine in this region of *c-bas/has*²⁷⁻²⁹ altering the *Msp*I recognition site at that location. Thus, *Msp*I should generate a 411-bp fragment of the T24 oncogene. In contrast, 355- and 56-bp fragments should be generated in the corresponding region of *c-bas/has* (human) (Fig. 4). To examine whether the Hs242 transforming sequence had an altered base within the sequence coding for amino acid 12, the Hs242 transforming gene, as well as cloned human *c-bas/has* and oncogenes, were digested with *Msp*I, blotted and hybridized with a DNA probe prepared from the purified 411-bp fragment of the T24 oncogene. Figure 4 shows that the Hs242 oncogene demonstrated a 355-bp DNA (lane 1) like that of *c-bas/has* (human) (lane 2), while the T24 oncogene yielded the expected 411-bp band (lane 3). Similar results were obtained with *Nae*I, an enzyme which can also recognize a sequence substitution for glycine in codon 12 (data not shown). These results indicated that the Hs242 oncogene had not undergone any alteration at amino acid position 12.

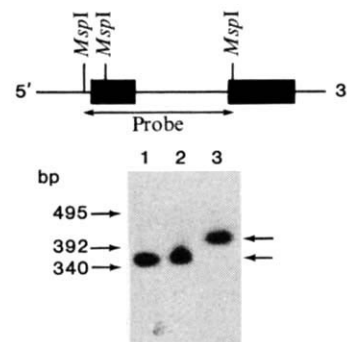


Fig. 4 Comparison of *Msp*I sensitivity at codon 12 of the Hs242 oncogene, *c-bas/has* (human) and the T24 oncogene. Upper panel shows the *Msp*I restriction map of *c-bas/has* (human). The T24 oncogene, which lacks the middle *Msp*I site, would yield the larger fragment as shown by the arrow. The two solid boxes indicate the first and second exons from the 5' to 3' direction. Lower panel shows Southern blot analysis of the Hs242 oncogene (pHs-49, lane 1), *c-bas/has* (human) (pbc-N1, lane 2) and the T24 oncogene (pT24-c3, lane 3). These DNAs were digested with *Msp*I, electrophoresed in a horizontal agarose (1.8% w/v) gel, blotted onto a nitrocellulose filter and hybridized to the T24 *Msp*I fragment as shown by the arrow in the upper panel. Co-electrophoresed DNA fragments of *Hinc*II-digested ϕ X174 RF DNA served as standards (labelled in base pairs). Migration of hybridized fragments is indicated by arrows. The *Msp*I 56-bp fragment was not observed in these experimental conditions.

We next sequenced the first exon of the Hs242 transforming gene; it exhibited a sequence identical to that of *c-bas/has* (human), over the entire stretch of 37 amino acids encoded by the first exon (Fig. 5). The Hs242 transforming gene, like *c-bas/has* (human), retained glycine at position 12 whereas the T24 oncogene contained valine (Fig. 5).

Genetic mapping of the lesion

To establish the region of the Hs242 oncogene that had undergone the genetic changes leading to its malignant activation, we constructed recombinants in which fragments of the Hs242 oncogene were substituted by the homologous sequences of *c-bas/has* (human). In reciprocal experiments, the same region of the *c-bas/has* (human) was replaced by the corresponding region of its transforming allele. Figure 6 shows the plasmids constructed in the present studies as well as their respective biological activities. Four types of recombinant plasmids were generated at the sites of *Sac*I cleavage (Fig. 6A, B). By transfection analysis, only plasmids containing the 2.9-kbp *Sac*I fragment of the Hs242 oncogene demonstrated transforming activity. Recombinant plasmids between *Xho*I and *Kpn*I sites (Fig. 6C) showed that the *Xho*I-*Kpn*I region was not responsible for activation of Hs242 DNA. This region was previously shown to contain the genetic alteration responsible for activation of the T24 bladder carcinoma oncogene²⁷⁻²⁹.

Finally, replacement of the *Kpn*I-*Nco*I region of *c-bas/has* (human) by the corresponding region of the Hs242 oncogene generated plasmids that transformed NIH 3T3 cells efficiently (Fig. 6D). In contrast, substitution within the Hs242 oncogene of the corresponding region of *c-bas/has* (human) completely abolished its transforming activity (Fig. 6D). These results localized the genetic alterations that activated the Hs242 oncogene within the 0.45-kbp region spanning the *Kpn*I to *Nco*I site.

Identification of mutation

We performed nucleotide sequence analysis on the 0.45-kbp *Kpn*I-*Nco*I DNA fragments of the Hs242 oncogene and its normal homologue to localize the lesion(s) responsible for its acquisition of transforming properties. The nucleotide sequences of the Hs242 oncogene and its normal allele are presented in Fig. 7. Sequence analysis revealed that the two

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
Hs242 oncogene	Met	Thr	Glu	Tyr	Lys	Leu	Val	Val	Val	Gly	Ala	Gly	Gly	Val	Gly	Lys	Ser	Ala	Leu	Thr	Ile	Gln	Leu	Ile	Gln	Asn	His	Phe	Val	Asp	Glu	Tyr	Asp	Pro	Thr	Ile	Glu
	ATG	ACG	GAA	TAT	AAG	CTG	GTG	GTG	GTG	GGC	GCC	GGC	GGT	GTG	GGC	AAG	AGT	GCG	CTG	ACC	ATC	CAG	CTG	ATC	CAG	AAC	CAT	TTT	GTG	GAC	GAA	TAC	GAC	CCC	ACT	ATA	GAG
c-bas/has	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	Gly	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	GGC	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
T24 oncogene	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	Val	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	GTC	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

Fig. 5 DNA sequence and predicted amino acid sequence of the entire first exon of the Hs242 oncogene. The previously reported sequences of *c-bas/has*(human) and the T24 oncogene²⁷⁻²⁹ are shown for comparison; dashes indicate sequences identical to those of the Hs242 oncogene except for codon 12 which is boxed. Nucleotide sequence analysis was performed according to the procedures of Maxam and Gilbert³⁸.

genes differed by a single base at position 150 (Fig. 7) within codon 61 of the p21 protein encoded by this gene. Figure 7 shows that an adenine residue in the *c-bas/has*(human) proto-oncogene has been replaced by thymine at this position, resulting in an alteration of the coding properties of this triplet. This change results in the incorporation of leucine instead of glutamine. Thus, a single amino acid substitution seems sufficient to confer transforming properties on the gene product of the Hs242 oncogene. These results also establish that the site of activation in the Hs242 oncogene is totally different from that of the T24 and EJ oncogenes.

Implications

A variety of human tumours and tumour cell lines have been shown to contain cellular oncogenes capable of transforming NIH 3T3 cells⁵⁻¹². Among such transforming genes thus far detected, a high proportion is related to a small family of retroviral *onc* genes. Oncogenes homologous to *c-kis* have been detected in tumours as diverse as carcinomas, sarcomas^{12,20} and haematopoietic tumours³⁹. Transforming genes related to *c-bas/has* have been detected previously in the human bladder carcinoma cell lines, EJ and T24^{10,20,24,25}. Our identification of a new transforming *c-bas/has* allele in a human lung carcinoma-derived cell line, Hs242, demonstrates that *bas/has*, like *kis*, can be activated in more than one tissue type.

The source of the oncogene described here was human cells derived from a tumour explant. Unlike other tumour cell lines, in which transforming DNA sequences have been detected⁵⁻¹², the Hs242 line was a mixed culture comprised predominantly of stromal cells of normal appearance. This may account in part for the 10–25-fold lower level of transforming activity associated with Hs242 cellular DNA compared with the DNA of other tumour cells⁷⁻¹⁰.

Recent studies on the molecular mechanisms involved in the activation of T24 and EJ bladder carcinoma-derived oncogenes have demonstrated the importance of a particular locus within *c-bas/has*(human) for the acquisition of transforming properties²⁷⁻²⁹. The genetic lesion has been localized to a point mutation in codon 12, where a guanine has been replaced by a thymine, resulting in the amino acid change from glycine to valine. Further evidence of the importance of this simple substitution has been derived from sequence analysis of the corresponding domains of Ha-, BALB- and Ki-MSVs. The first 37-residue amino acid sequence encoded by each of these *onc* genes is almost identical to the first exon of the human cellular *c-bas/has* allele²⁷⁻²⁹, despite the fact *v-bas* and *v-has* are of mouse and rat origin, respectively, and *v-kis* originated from a different proto-oncogene and is also of rat origin. Yet, at

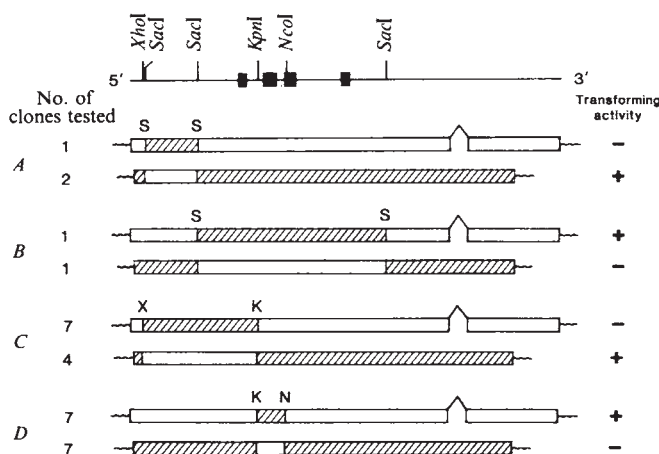


Fig. 6 Location of the region of *c-bas/has*(human) that underwent the genetic alterations which led to activation of the Hs242 oncogene. The restriction map shows the cleavage sites for various enzymes within the insert in pBR322. Hybrid plasmids contained sequences from the Hs242 oncogene (pHs-49; cross-hatched regions) and *c-bas/has*(human) (pbc-N1; open regions). Constructs were made in A and B by mixing the three fragments simultaneously, and in C and D by bimolecular ligations. Replaced fragments were *SacI* 0.8 kbp (A); *SacI* 2.9 kbp (B); *XhoI*-*KpnI* 1.8 kbp (C); and *KpnI*-*NcoI* 0.45 kbp (D). Deletion (Δ) in pbc-N1 and the locations of the four exons (solid boxes) are depicted. The left-hand column shows the number of independent clones transfected onto NIH 3T3 cells. Results of the transfections are shown at the right-hand side: +, $>10^3$ FFU per μ g; -, $<10^0$ FFU per μ g.

position 12 each *v-onc* has substituted a different amino acid: that is, lysine (E.P.R. *et al.*, in preparation), arginine¹⁸, and serine¹⁷, respectively. These findings have led to the speculation that the target for oncogenic conversion may be exceedingly small and may even be confined to the glycine codon at position 12 (refs 27–29).

In the present studies, we molecularly cloned the Hs242 oncogene. This transforming gene was shown to possess a specific transforming activity for NIH 3T3 cells rivaling that of the most potent retroviral *onc* genes. Our isolation of this gene has allowed us to compare directly the mechanisms by which the *c-bas/has*(human) proto-oncogene can be independently activated as a transforming gene in natural conditions. By construction of recombinants between the Hs242 oncogene and its normal allele, we localized the region responsible for activation of this transforming gene to a 450-bp segment span-

	50	100
GGTACCAGGAGGCTGGCTGTGTGAACCTCCCCACGGAAGGTCTGAGGGGTCCTGAGCCCTGCTCCTGCAGGAT		TCC TAC CGG AAG CAG GTG
		Asp Ser Tyr Arg Lys Gln Val
		Splice
GTC ATT GAT GGG GAG ACG TGC CTG TTG GAC ATC CTG GAT ACC GCC GGC CTG GAG GAG TAC AGC GCC ATG CGG GAC CAG TAC		
Val Ile Asp Gly Glu Thr Cys Leu Leu Asp Ile Leu Asp Thr Ala Gly Leu		Glu Glu Tyr Ser Ala Met Arg Asp Gln Tyr
		Splice
ATG CGC ACC GGG GAG GGC TTC CTG TGT GTG TTT GCC ATC AAC AAC ACC AAG TCT TTT GAG GAC ATC CAC CAG TAC AGG TGA		
Met Arg Thr Gly Glu Gly Phe Leu Cys Val Phe Ala Ile Asn Asn Thr Lys Ser Phe Glu Asp Ile His Gln Tyr Arg		
		Splice
ACCCCGTGGAGCTGGCCGGGAGCCCAAGCCGACAGGTGGGCGGACGCCGCTGCCTCCAGGAGGGGCTCTCTCTCTCGCATGTCTGGATGCCGCTGC		
		Splice
GCCTGCAGCCCCCGTAGCCAGCTCTGCTTTCACCTCTCAGG		GAG CAG ATC AAA CGG GTG AAG GAC TCG GAT GAC GTG CCC ATG GTG
		Glu Gln Ile Lys Arg Val Lys Asp Ser Asp Asp Val Pro Met Val

Fig. 7 Comparison of the DNA sequence and predicted amino acid sequence between the *KpnI* and *NcoI* sites of the Hs242 oncogene and *c-bas/has*(human). A single base change (A to T) and the consequent amino acid change of glutamine to leucine are boxed. *KpnI* and *NcoI* cutting sites (∇) and splicing sites ($\downarrow$) are also shown.

ning the second exon and a small portion of the third exon. Comparative nucleotide sequence analysis further localized the lesion affecting the Hs242 oncogene to codon 61. A point mutation in this codon arising from the transversion of A to T results in the incorporation of leucine instead of glutamine at this position. Thus, a single amino acid substitution not just at one site, but even within different regions of the molecule, seems to be sufficient for the p21 protein to acquire transforming properties.

The Hs242 oncogene-encoded p21 protein exhibited an altered electrophoretic mobility. Preliminary sequence analysis of the remainder of the coding region has revealed no evidence of additional genetic changes, arguing that the altered electrophoretic mobility is a direct result of the amino acid substitution at position 61. These results imply that the Hs242 oncogene product has suffered a conformational change as a result of the point mutation. A conformational change in the molecule has also been ascribed to the mutation affecting the T24 oncogene²⁷⁻²⁹.

Recent evidence has indicated that *c-bas/has* (human) can also acquire transforming properties when its coding region is placed under the influence of the strong promoter sequence signals of a retroviral long terminal repeat (LTR), implying that elevated levels of the normal p21 gene product can also induce cell transformation²⁶. Our present findings argue that by whatever mechanism the altered genes disrupt regulation of cellular growth, qualitative rather than quantitative alterations are probably the most common cause of the gene acquiring transforming properties in natural conditions.

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Studies on the evolutionary divergence of genes have made it possible to estimate the rate of change of synonymous codons. Frequencies in the order of $6-7 \times 10^{-9}$ substitutions per nucleotide site per yr have been independently calculated for synonymous substitutions in genes such as that for preproinsulin and globin^{31,32}. During the first year of human life, with an average population of 10^{12} cells at risk, it can be estimated that base substitutions affecting codons at either of the established sites of Hs242 or T24 oncogene activation might occur in thousands of cells. While not all such base changes might be expected to lead to amino acid substitutions capable of activating the gene, certainly many such events would be expected. It is known that acute transforming retroviruses, which have transduced closely related *onc* genes from mouse or rat, can rapidly induce a variety of tumours *in vivo* as well as transform cells within several developmental pathways in tissue culture^{33,34}. Thus, if the closely related Hs242 and T24 oncogenes were equally potent in their ability to transform normal diploid cells, the predicted spontaneous frequency of lesions at critical sites within the *c-bas/has* (human) proto-oncogene might be expected to lead to a more rapid onset of cancer in natural conditions. Further studies will be needed to resolve this apparent paradox.

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LETTERS TO NATURE

Why is M87 jet one sided in appearance?

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The jet in M87 is one sided and knotty. The question of whether the one sidedness is due to relativistic beaming or to intrinsic one sidedness has not yet been resolved. Recently, the knots have been observed in detail at the Very Large Array (VLA)¹; knot A is observed to consist of a thin disk, apparently a shock that is perpendicular to the jet axis and viewed edge-on, where the radio brightness profile has a very sharp gradient, and also of a trail downstream of the disk, that is pointing away from the nucleus. We discuss here the geometric implications of these observations, in particular the sharp edge facing the nucleus. We discuss models that represent the extremes in the apparent motion of the shock, and argue that proper motion measurements of the knot's inner edge would discriminate between them.

The knots may be shocks caused by 'fast streams' in the jet², analogous to co-rotating shocks in the solar wind, or the repeated cycles of the underexpansion and overexpansion of the jet material^{3,4}. In the fast stream model, the knots would be expected to move with the jet material; in the latter model, they would most likely be stationary if the jet is in a hydrodynamic steady state. Also, if the shock is caused by a giant stationary obstacle⁵, or the entrainment of denser material via instabilities, it is likely to be stationary or slowly moving. If it is a standing shock in the flow, it is truly edge-on. However, the relativistic beaming hypothesis for explaining the one sidedness combined with the fast stream model for the knots also allows the shock to appear edge-on, and as shown below, it predicts a minimum proper motion that is much higher than previous estimates.

It is clear that a shock produced by a fast stream would probably be perpendicular to the jet axis. If it is caused by the convergence of overexpanded material, a shock that is perpendicular to the axis (a Mach disk) may or may not form depending on the extent to which the jet is collimated on compact scales. If it is initially well collimated, the shocks that form where the material converges downstream intersect at a point and are

quasi-parallel to the jet axis, so that the jet material passes through them obliquely and remains supersonic. If the material diverges from its source with a wide opening angle, it converges downstream at a comparably wide angle, much of the bulk energy is converted to heat, and the thermal pressure at the point of convergence is comparable with the ram pressure along the jet axis. The pre-shock material near the axis must then encounter the shocked material squarely, meaning that a shock has formed perpendicular to the axis. This basic picture is consistent with the laboratory data of Love *et al.*⁶ that show Mach disk formation to be positively correlated with opening angle and jet overpressure at the nozzle, and inversely correlated with Mach number.

Knot A is ~ 1 kpc from the nucleus if the jet is in the plane of the sky. The ambient pressure 3×10^{-10} dyn cm $^{-2}$ inferred by X-ray observations⁷ is enough to focus beams over this length if (L/v) the ratio of beam power to velocity is $< 10^{33}$ erg cm $^{-2}$ (ref. 8). Optical filaments in the M87 nucleus show evidence for a higher pressure⁹, which would allow a larger L/v .

We shall tentatively work with the hypothesis that the jet has axial symmetry and that the shock is a disk that is perpendicular to the axis. Clearly, if the motion of the shock is non-relativistic relative to the observer, the jet must be oriented perpendicular to the observer's line of sight to be viewed edge-on. If the jet and shock within it move relativistically this need not be the case since light travel time effects will cause an apparent rotation of the shock plane. Light emitted from the part of the shock furthest from the observer takes longest to reach the observer. To arrive at the same time as light from the near part of the shock requires that it be emitted earlier. The delay in seeing the far side gives rise to the rotation. As a result, a relativistic shock in a jet pointing in the plane of the sky will not be seen edge-on. Thus, if the M87 jet lies in the plane of the sky, the shocks are not moving relativistically. In projection, a circular disk moving non-relativistically appears as an ellipse, with its major axis perpendicular to the jet and with an ellipticity (the ratio of axes) $\eta = \cos \phi$, where ϕ is the angle between the jet axis and the line of sight. The probability that $\eta < \eta_1$ for a randomly directed jet is simply η_1 . From the observations η appears to be low for M87, although not implausibly so ($\eta \lesssim 0.2$).

The shock will appear edge-on if $v_s/c = \cos \phi$ where v_s is the shock velocity in the observer's frame. This result can be easily derived by considering the configuration at hand (Fig. 1). The shock line AB will appear to the observer as AB' when the time taken by the shock to move from B' to B is equal to the light travel time from B' to A, that is, $(BB')/v = (AB')/c$, where $BB'/AB' = \cos \phi$. Expanding $\cos \phi$ as $1 - \phi^2/2$, it is easily shown that an ultra-relativistic shock moving at an angle $1/\gamma$ to the line of sight will appear edge-on to lowest order in ϕ (or $1/\gamma$), where γ is the Lorentz factor. Exact solutions are contained in Fig. 2 which plots as a function of ϕ the apparent ellipticity η of a shock disk moving along its axis for various speeds¹⁰.

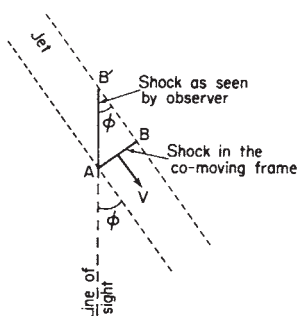


Fig. 1 The basic geometry that defines ϕ is shown.

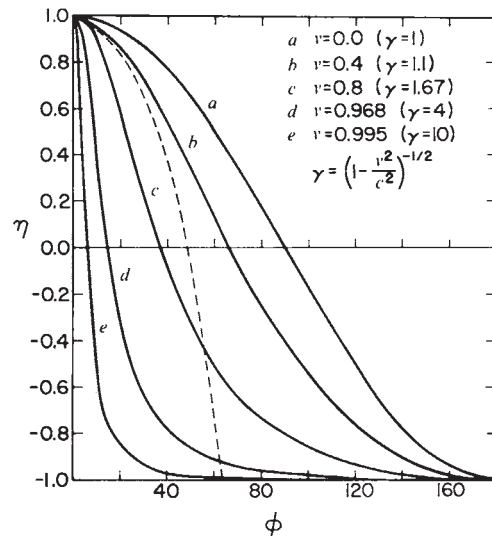


Fig. 2 The apparent ellipticity η (y axis), as seen by an observer, is plotted for a disk moving along its axis at angle ϕ (x axis) to the line of sight, for various values of disk velocity. The region left of the dotted line allows relativistic beaming for the M87 jet.

Shocks are likely to develop in a beam from internal irregularities in velocity structure of order $\Delta v/v \approx M^{-1} \gamma^{-2}$. To retain good collimation, $\Delta v/v$ is probably small. The shocks and jet material move at the same speed to zero-th order in $\Delta v/v$, and the difference between v_1 , the velocity of the shocked material, and the shock velocity v is further reduced by a factor of $\sim 1/\zeta$, where ζ is the compression ratio of the shock. Since the tail of diffuse emission is on the opposite side of the galactic nucleus from the sharp edge, we presume that a reverse shock would be responsible. Without given initial conditions the manner in which shocks form cannot be calculated. Thus we cannot comment on the absence of a sharp boundary from a forward shock.

Relativistic beaming is a valid explanation for the non-appearance of an oppositely directed jet provided $u/c \cos \phi > 0.45$ where u is the average velocity of the jet¹⁰. This condition is satisfied by the region left of the dotted line in Fig. 2 for the case $u = v_s$. Velocities < 0.45 are ruled out immediately, and assuming $\eta < 0.2$, $v \approx 0.6c$. (We infer from this diagram that the shocks in the unseen jet would appear almost face-on if the jets are intrinsically mirror symmetric about the nucleus.) There is no *a priori* reason to have $\phi = \cos^{-1} v/c$. The probability for $|\eta| < 0.2$ in the case $v/c = 0.8$ with $(v/c) \cos \phi > 0.45$, and ϕ otherwise randomly distributed, is 0.35. At higher v/c the probability decreases (reaching 0.01 for $\gamma \approx 1.5$), however, selection effects favour small ϕ for ultra-relativistic beams, so significantly higher γ does not render $\phi \approx 1/\gamma$ implausible.

In this model, the fact that the shock is seen edge-on proves fortunate since the edge-on criterion is also the criterion for the maximum apparent expansion velocity, v_{app} , for a given ϕ . This velocity then implies $v_{app} = c \cot \phi$ and is > 1 for $\phi < 45^\circ$ (ref. 9). This converts to a minimum of 3.7 m arc s yr $^{-1}$, which, being an order of magnitude larger than previous estimates¹¹, should be measurable within a decade or so.

If the shock is not exactly perpendicular to the jet axis, the above estimate for minimum proper motion is not exact. In the relativistic beaming model, much of the $\sim 15^\circ$ deviation observed for knot A can be attributed to a time-delay effect: the intrinsic angular deviation from a perpendicular shock appears to be amplified by a factor $\csc \phi$, and it therefore seems likely that the intrinsic tilt of the shock away from the perpendicular is small in the relativistic beaming model. Therefore, $v_s/c \approx \cos \phi$ and the edge-on appearance of the shock constrains the proper motion to a minimum that is approximately the value quoted here. (Of course, the minimum is only a probable one; it can in any case be invalidated by an improbable geometry.)

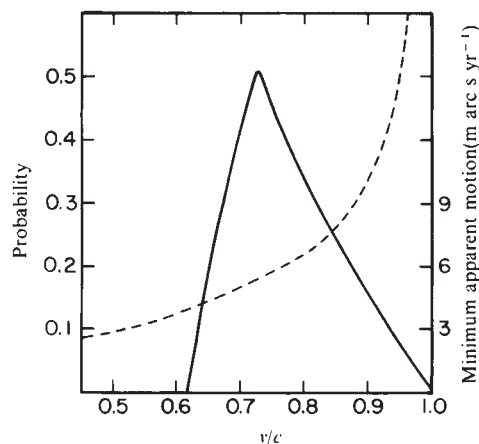


Fig. 3 The probability is plotted as a function of v_s that a shock in the M87 jet will appear approximately edge-on ($\eta < 0.2$) given that the one sidedness is due to relativistic beaming and assuming that the jet angle is otherwise random (left axis). Also plotted (right axis) is the minimum apparent motion as a function of v_s given that $\eta < 0.2$ (dotted line).

If the shock is not a flat disk, it seems even less likely that it would appear as a sharp edge.

We have discussed models for M87 that are viable accounts of the sharp, edge-on structures in its radio jet. The models predict opposite extremes of apparent shock motion, one class invoking a more or less stationary Mach shock disk and the other invoking a relativistic shock with the maximum possible apparent velocity for a given angle to the line of sight. The standing (or non-relativistic) shock model requires that the jet be intrinsically one sided, for a standing shock with relativistically beamed post-shock emission would not appear edge-on. The other model, which invokes relativistic beaming, requires that $v_s/c > 0.6$ and $\phi \approx 1/\gamma$, so that a minimum apparent proper motion of ~ 4 m arc s yr $^{-1}$ is predicted. While neither model is complete, there appears to be a well defined observational test to distinguish between them and, dismissing improbable geometric coincidence, thereby to resolve the question of why the jet appears one sided.

Note that M. Reid (personal communication) informs us that second epoch very long baseline interferometry observations at 18 cm, still in the data analysis stage, show no obvious change in the nuclear region over 2 yr. It is reasonable to assume that the jet has the same orientation and flow velocity on nuclear and kpc scales. Hence, given their resolution of 8 m arc s, they are on the verge of producing strong evidence against the relativistic beaming model. It remains a logical possibility, of course, that the nuclear knots are produced by a stationary obstacle even in the relativistic beaming model, so it still seems desirable to look for proper motion in the same knot that shows the sharp edge. Sulentic *et al.*¹² claim an upper limit of 5 m arc s yr $^{-1}$ having compared photographic plates taken 22 yr apart.

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Do pulsating PG1159–035 stars put constraints on stellar evolution?

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The variable star PG1159–035, discovered by McGraw *et al.*¹, is the prototype of a new class of extremely hot pulsating degenerate stars. Here we present preliminary theoretical results indicating that the pulsation periods of these stars should be decreasing with an e -folding time of the order of 10^6 yr—a value directly reflecting their rapid cooling times. We point out that such a rapid change in period should be measurable on a time scale of 1–3 yr, and that such measurements should be a direct test of stellar evolution and pulsation theory for hot degenerate objects.

McGraw *et al.* demonstrated¹ that PG1159–035 was multiperiodic with at least two pulsation periods of ~ 540 and 460 s simultaneously present in the light curve. Optical and IUE spectrophotometry, coupled with Einstein and Voyager 2 data, indicates an effective temperature $T_e \sim 120,000$ K ($+50,000$ – $20,000$), and a gravity $7 < \log g < 8$ (J. T. McGraw, personal communication). Five additional objects have been identified^{2,3} with strikingly similar spectra, showing that PG1159–035 is a member of a spectroscopic class and is not a pathological object. Of these five, two were examined for variability and both were observed to pulsate with periods similar to that of PG1159–035 (ref. 3). The evolutionary state of these objects appears to be intermediate between stars similar to the hot nuclei of planetary nebulae and their final end products, the white dwarfs.

Theoretical pre-white dwarf evolutionary calculations include the early work of Savedoff and collaborators^{4,5}. More recently, Winget, Lamb and Van Horn (in preparation) have computed the evolution of carbon pre-white dwarfs using state-of-the-art physics. In these models, the rate of neutrino emission has been calculated using the older results of Beaudet *et al.*⁶; the newly established⁷ mass of the W intermediate boson (~ 81 GeV) validates the older rates to within $\sim 10\%$ (ref. 8). Neutrino emission for hot, degenerate objects is important in controlling the rate of evolution and, for models in the T_e and g range of PG1159–035, the resulting neutrino luminosity is a factor of 2–5 greater than the photon luminosity. Neutrino cooling produces a temperature inversion in the core, with a broad flat maximum that moves outwards in the models as they cool towards the white dwarf region.

Recently, Starrfield *et al.*⁹ have computed the pulsation properties of non-evolutionary models in the T_e and g range of PG1159–035. Their work indicates that the pulsations may be identified with nonradial gravity modes of fairly high overtone and low spherical harmonic index. This suggests the possibility of measuring the rate of period change, $\dot{P}$, using the techniques developed by Kepler *et al.*¹⁰ for the ZZ Ceti variable white dwarfs. In turn, the observed $\dot{P}$ can be used in conjunction with the theoretical models to infer evolutionary time scales for these stars.

We can estimate the expected $\dot{P}$ s on the basis of simple analytical considerations and the results of the evolutionary calculations. (A complete and detailed analysis is in progress.)

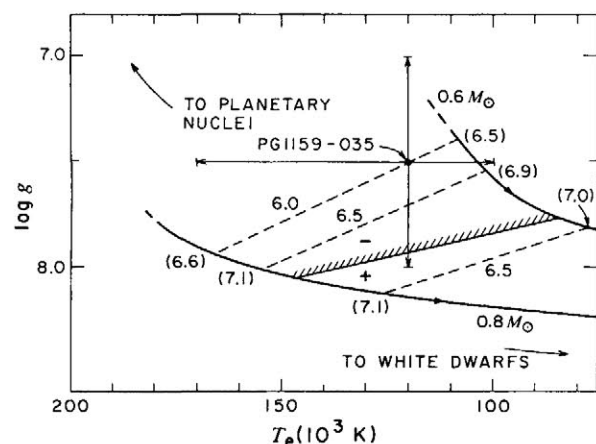


Fig. 1 The error box of PG1159-035 is displayed on the T_e - $\log g$ plane along with evolutionary tracks of $0.6 M_\odot$ and $0.8 M_\odot$ cooling white dwarfs. The approximate magnitude and sign of period rates of change are indicated.

For high overtone g (gravity) modes we can write¹¹:

$$\Pi_g^2 \approx -\frac{k^2 r^2}{A g l(l+1)} \quad (1)$$

where

$$A = \frac{\chi_T}{\chi_\rho} (\nabla - \nabla_{ad}) / H_p, \quad \chi_T = \left(\frac{\partial \ln P}{\partial \ln T} \right)_\rho$$

$$\chi_\rho = \left(\frac{\partial \ln P}{\partial \ln \rho} \right)_T \quad (2)$$

Here, k is the radial wavenumber, l the spherical harmonic index, g the local gravity at radial position r , ∇ is the actual stellar temperature gradient, ∇_{ad} is the adiabatic temperature gradient, and H_p is the pressure scale height. Equation (1) applies more to high overtone modes but is adequate for our purposes. If we assume that the period is determined in the region where the broad temperature maximum occurs¹², then the approximation that ∇ is small is a reasonable one. (This is true also for models in which neutrinos are neglected since the cores of such models tend to be isothermal.) With this approximation we can differentiate equation (1) with respect to time, t , and find

$$\frac{\dot{\Pi}}{\Pi} \approx -\frac{1}{2} \frac{\dot{T}_m}{T_m} + \frac{\dot{R}}{R} \quad (3)$$

Here, T_m is the temperature maximum and R is the stellar radius. If we use equation (3) to estimate $\dot{\Pi}/\Pi$ for cool white dwarfs we find agreement with the results of detailed pulsation calculations to within 30%. In this instance the logarithmic change in radius is small (in absolute value) compared with $\dot{T}_m/T_m < 0$ since cool white dwarfs evolve at essentially constant radius and hence periods increase with time. However, for hot models, which presumably represent PG1159-035, the opposite may be true. This situation is displayed in Fig. 1 where the evolutionary tracks of $0.6 M_\odot$ and $0.8 M_\odot$ cooling proto-white dwarf models are shown in the T_e - $\log g$ plane along with the location of PG1159-035 with appropriate error bars. The solid line connecting the two tracks marks a change in sign for $\dot{\Pi}$ and the dashed lines indicate lines of constant $\dot{\Pi}$ with the value of $\log |\dot{\Pi}/\Pi|$ written above the line ($\dot{\Pi}/\Pi$ here has the units of years). The same quantity for the case of no neutrino losses is given in parentheses.

Our preliminary results allow several interesting conclusions. First, even with the present rather large uncertainties in the temperature and gravity of PG1159-035, we conclude that $\dot{\Pi}$ is probably negative. Although our models assume a pure carbon core, the presence of elements heavier than carbon

would only delay the effects of electron degeneracy slightly, and move the positive-negative boundary to lower temperatures. Thus, as opposed to other pulsating degenerates such as the ZZ Ceti stars, we expect the periods of PG1159-035, and probably the others in its class also, to be decreasing. Further, the order of magnitude of the time scale for period changes indicated by our results, $|\dot{\Pi}/\Pi| \sim 10^6$ yr, implies that the period change can be measured on a time scale of one to three observing seasons depending on the complexity of the light curve (E. L. Robinson, personal communication).

It now seems possible that the course of evolution of PG1159-035 and other members of its class may be inferred by observation of their pulsation characteristics even though evolutionary time scales are of the order of millions of years. This would, of course, also be a measure of the internal constitution of such stars. Thus, such a combination of observation and theory would, in conjunction with like efforts for the ZZ Ceti variable white dwarfs, constitute a significant advance in our understanding of white dwarf progenitors and their evolution. In addition, sufficiently precise measurements of $\dot{\Pi}$ may confirm the validity of astrophysical applications⁸ of electro-weak theory to neutrino emission from hot, dense plasmas, and, more speculatively, to perhaps set more stringent limits on the mass of the recently hypothesized axion¹³⁻¹⁵.

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Albedo asymmetry of Iapetus

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Voyager images of Saturn's moon, Iapetus^{1,2}, confirm deductions made from Earth-based observations dating back to 1671 of a very dark leading hemisphere and a very bright trailing hemisphere³⁻⁵. Figure 1 displays contours of surface albedo from three Voyager images. The darkest area is at the apex of orbital motion, with a pronounced ($\sim 10\times$) increase in albedo towards the antapex, constituting the greatest interhemispheric albedo contrast known in the Solar System. The poles are brighter still. Figure 1 also shows that the albedo distribution resembles the calculated areal variation of the trans-saturnian impact flux⁶ remarkably closely. Dark areas correspond to regions with the highest calculated flux. We propose here that

the dark areas contain organic chromophores produced *in situ* by UV irradiation of CH_4 -rich ice, and that the albedo pattern results from ballistic redistribution of surface material in response to the impact flux gradient. Where the impact flux is high, net ablation will cause exposure of CH_4 -rich darkenable ice, creating a dark surface. Where the flux is low, net accumulation of non-darkenable icy regolith that has lost CH_4 through repeated impact volatilization and evaporation, will create a bright surface.

The surface of Iapetus is heavily cratered. While there is at least one dark-floored impact crater on the bright trailing hemisphere (Fig. 2), no bright-floored craters are apparent on the dark leading hemisphere. Voyager colour images show Iapetus to be spectrally red, in agreement with Earth-based measurements⁷. We measure typical orange/violet ratios for Voyager colour filters (centred near 0.59 and 0.42 μm , respectively) as 1.46 for the leading hemisphere and 1.24 for the trailing hemisphere. For comparison, Phoebe, a small dark satellite in a retrograde orbit beyond that of Iapetus, has a mean orange/violet ratio of 1.05. Phoebe's near-IR spectrum is also different from that of either hemisphere of Iapetus⁷.

Several hypotheses have been proposed to account for the albedo asymmetry of Iapetus. Cook and Franklin⁶ suggested impact erosion of a thin ice veneer from the leading hemisphere of a dark essentially silicate body. But Voyager measurements² show the mean density of Iapetus to be $1.16 \pm 0.09 \text{ g cm}^{-3}$, typical of ices, not silicates. Soter⁸ proposed that the leading hemisphere is coated with dark material ejected from Phoebe by impacts and dragged towards Saturn by the Poynting-Robertson effect. This hypothesis is inconsistent with the large colour differences between Iapetus and Phoebe (unless dramatic changes in optical properties accompany ejection and transport) and with the presence of dark-floored craters on the trailing hemisphere. Smith *et al.*² suggested that dark carbonaceous material has been extruded to the surface preferentially on the leading hemisphere. However, for an eruptive pattern to match so closely the isopleths of the meteorite flux would require a coincidence of remarkable proportions.

Because no external source of appropriate dark reddish material is known, a successful hypothesis must posit darkening of ices *in situ*. The dense N_2/CH_4 atmosphere of Titan implies that NH_3 and CH_4 were incorporated in that body as it formed. The material making up Iapetus condensed at still greater distances from the luminous proto-Saturn, and presumably at still lower temperatures⁹. It is likely, then, that Iapetus contains significant amounts of methane and ammonia, as $\text{CH}_4 \cdot x\text{H}_2\text{O}$ and $\text{NH}_3 \cdot \text{H}_2\text{O}$. We propose that the dark reddish material on Iapetus is composed of organic chromophores generated from CH_4 -rich ice.

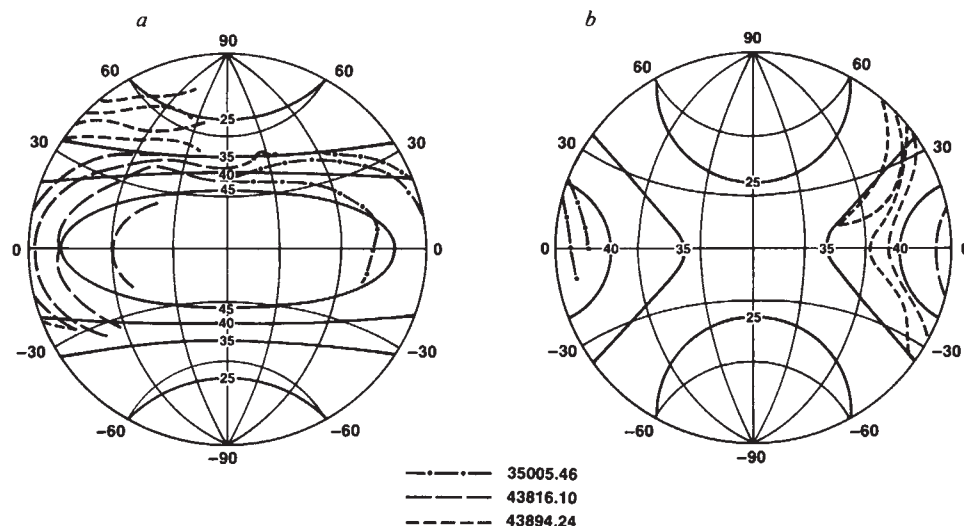
The evolution of Iapetus' regolith depends on the relative rates of several processes: ice evaporation, impact ablation,

material transport, and chemical alteration. At the heliocentric distance of Saturn, ices of H_2O and $\text{NH}_3 \cdot \text{H}_2\text{O}$ are essentially stable, while the evaporation rate E of $\text{CH}_4 \cdot x\text{H}_2\text{O}$ is high¹⁰ ($\sim 0.2 \text{ cm yr}^{-1}$). A mixture of these ices will therefore lose CH_4 from its surface and be at least partially replenished by diffusion of CH_4 from below. Ices of all types will gradually be ablated, chiefly by impact volatilization and subsequent loss to space. But because $\text{CH}_4 \cdot x\text{H}_2\text{O}$ is much more volatile than the other ices, the net result of many impacts will be a major CH_4 depletion in any accumulated regolith.

There will also be a net ballistic transport of material from regions of high impact flux to regions of low impact flux. Using the meteoritic mass flux at Earth¹¹ scaled to the expected flux at Saturn², we estimate a mean mass flux $\bar{J}_t \sim 3 \times 10^{-11} \text{ g cm}^{-2} \text{ yr}^{-1}$ striking Iapetus from outside the Saturn system. The actual trans-saturnian flux will vary by roughly a factor of 2 from leading to trailing hemisphere⁶ (see Fig. 1), and will also be augmented on the leading hemisphere by material from Phoebe. Because of the outer satellite's retrograde orbit, encounter velocities with debris from Phoebe will be high ($\sim 6.5 \text{ km s}^{-1}$), comparable to the velocity of material arriving from outside the system (the escape velocity from Saturn at Iapetus' orbit is $\sim 4.5 \text{ km s}^{-1}$). The mass flux of material striking Iapetus' leading hemisphere is $J = (4\pi R_p^2 \bar{J}_t f \psi_p \gamma / 2\pi R_i^2) + J_t$, where R_p and R_i are the radii of Phoebe and Iapetus, f is the ejecta/projectile mass ratio, ψ_p the mass fraction of ejecta that escapes Phoebe, and γ the fraction of escaped ejecta eventually reaccreted by Iapetus. Typical values of f from hypervelocity cratering experiments¹²⁻¹⁴ are 10^3 – 10^4 . The value of ψ_p depends sensitively on the surface strength of Phoebe, and could range from 0.2 for coherent material to 4×10^{-5} for zero-strength material¹⁵. Taking the maximum probable values ($f = 10^4$, $\psi_p = 0.2$, $\gamma = 1$), $J_{\text{max}} \sim 2 \times 10^{-9} \text{ g cm}^{-2} \text{ yr}^{-1}$. The minimum flux for the leading hemisphere is the trans-saturnian flux adjusted for Iapetus' orbital motion, $J_{\text{min}} = J_t \sim 4 \times 10^{-11} \text{ g cm}^{-2} \text{ yr}^{-1}$. The variation in the impact flux from leading to trailing hemisphere may therefore be as little as a factor of 2 or as much as a factor of 100.

Iapetus' low gravitational acceleration allows impact ejecta to travel large distances. Particles on the leading hemisphere have a high probability of being ejected and landing on the trailing hemisphere, but, because of the lower impact flux on the trailing hemisphere, a smaller probability of being returned by impacts to the leading hemisphere. The impact flux gradient across the body will therefore result in a net ablation of material from the leading hemisphere, and a net accumulation of impact ejecta on the trailing hemisphere. The absolute rates of ablation and deposition depend in a complex fashion on the fallback pattern of the ejecta (which in turn depends sensitively on impact velocity and regolith cohesion) and on the spatial variation of the impact flux. By calculating ejecta trajectories and

Fig. 1 Distribution of albedo on Iapetus on leading (a) and trailing (b) hemispheres. Three different sets of dashed contours connect points of equal reflectance from three different Voyager images, with the photometric function of the material removed. The darkest area is centred at the apex of orbital motion. The contour interval varies among the three images. Some of the inflections in the photometric contours are due to local topography. Solid contours give the distribution of calculated trans-saturnian impact flux in arbitrary units⁶.



integrating the ejecta distribution over the surface of the satellite¹⁶, we estimate a net erosion rate of up to $4 \times 10^{-7} \text{ g cm}^{-2} \text{ yr}^{-1}$ (16 m over 4,000 Myr) at the apex.

UV irradiation of water ice with simple organic ices at Iapetus ambient temperatures ($\sim 77 \text{ K}$) yields a variety of more complex organic products¹⁷; despite the low temperature there is substantial free radical mobility. When mixtures of H_2O , NH_3 and carbon-rich ices (CH_4 and CO) are subjected to UV irradiation^{18,19}, a variety of still unidentified organic solids are produced, some of which absorb strongly in the visible. Judging from experiments in the gas phase²⁰, there are several visible wavelength organic chromophores that might be expected to form on prolonged UV irradiation of $\text{CH}_4/\text{NH}_3/\text{H}_2\text{O}$ ices on Iapetus, including conjugated polyenes, polyaromatic hydrocarbons and porphyrins. A series of experiments that closely approximate expected conditions on Iapetus is needed to determine whether the spectral characteristics of the organics produced match those of Iapetus.

Because Iapetus lies outside the saturnian magnetopause, we neglect irradiation of ices by magnetospheric particles. The UV every flux even at comparatively short wavelengths significantly exceeds that from cosmic rays and the solar wind. We consider an ice surface of mean molecular weight μ exposed to a solar UV flux of absorbed photons, F . Organic chromophores are generated with quantum yield ϕ , and the material is considered darkened when a fraction β of the chemical bonds are converted to chromophore bonds. The UV darkening saturation rate is then $R = \mu m_H \phi F / 2\beta$, where m_H is the mass of a hydrogen atom. For UV darkening to keep pace with a reasonable ballistic ablation rate Q (that is, $R = Q$), plausible values of the quantum yield are implied. For example, for $\lambda < 1,450 \text{ \AA}$, $\mu = 10$, $\beta \sim 10^{-3}$, and $Q \sim 10^{-7} \text{ g cm}^{-2} \text{ yr}^{-1}$, $\phi \sim 10^{-4}$. Roughly comparable values of ϕ/β are implied by published experiments^{18,19}. In all cases $E \gg R$ and $E \gg J$.

We consider first a model in which a thick dark layer is generated very early in Iapetus' history by, for example, hypervelocity impacts or solar UV radiation. Shock waves in simple reducing gases are known to generate more complex organic molecules with high efficiency²¹. UV darkening would have to be accompanied by efficient deep mixing for the resulting dark layer to be adequately thick.

To account for the appearance of Iapetus, the thick dark layer must have been covered by a stratum of bright material that was later eroded from the leading hemisphere. This stratum must be at least $\sim 10 \text{ km}$ thick, as impacts forming craters many tens of kilometres in diameter on the trailing hemisphere have not in general excavated into dark material. Substantial topography on the initial dark surface must be invoked to explain how apparently only one crater on the trailing hemisphere (and not one of the largest) has excavated through the putative bright stratum, creating a dark floor. Also, the bright stratum must have formed very early, since it shows, on the trailing hemisphere, a record of extensive cratering. It must have been free of CH_4 , so that subsequent bombardment and UV irradiation would not have turned it dark also. The most likely means for forming such a stratum would be vulcanism in which a $\text{H}_2\text{O}/\text{NH}_3/\text{CH}_4$ liquid magma was extruded to the surface and outgassed all its CH_4 before freezing. Evidence exists for some resurfacing after completion of heavy bombardment on other saturnian satellites^{1,2}.

If an initially uniform depth of the thick bright stratum is assumed, then $\sim 10 \text{ km}$ of it must have been removed by impact erosion from the leading hemisphere to re-expose the underlying dark material. The implied mean ablation rate since the end of heavy bombardment would therefore be $\sim 3 \times 10^{-3} \text{ g cm}^{-2} \text{ yr}^{-1}$. Even if we make the unreasonably generous assumption that 100% of the ejecta produced by impacts on the leading hemisphere was permanently lost, and take $f = 10^4$, our calculated maximum meteoritic mass flux for the leading hemisphere gives an erosion rate too small by a factor of ~ 20 . The apparent lack of an adequate erosion rate and the necessity of invoking a major resurfacing event for which there is no direct

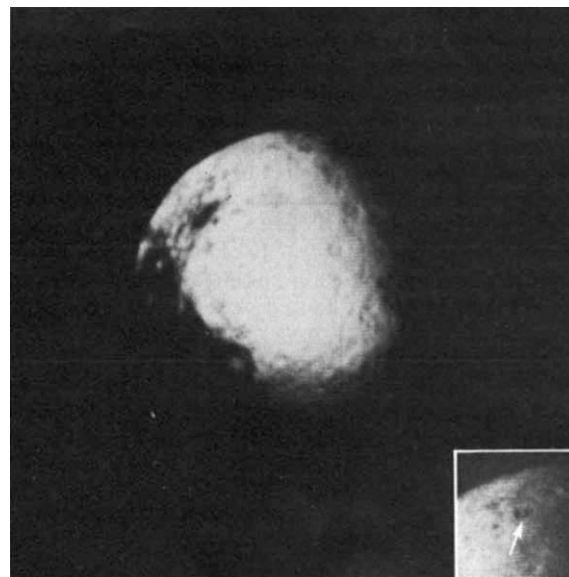


Fig. 2 Voyager 2 images of Iapetus, showing the dark leading hemisphere, bright trailing hemisphere, and a dark-floored crater (inset).

evidence lead us tentatively to reject this hypothesis of an early thick dark layer. If impact processes drove the darkening, a deep primitive dark layer would be expected, so that rejection of the hypothesis of an early dark layer implies rejection of impact-induced darkening. We also point out that Hyperion, if it is an icy body, has an appearance inconsistent with this hypothesis. It lies outside the orbit of Titan, so we expect it to contain CH_4 , and its irregular shape precludes the possibility of a major resurfacing event. We therefore would expect it to have an albedo comparable with that of Iapetus' leading hemisphere (< 0.05) if the hypothesis were correct. Its albedo¹ is, however, considerably higher (~ 0.25).

We now consider a model in which a thin dark layer is maintained on the leading hemisphere by a steady state between UV photochemistry and impact ablation as, we have argued, can be accomplished with plausible quantum yields. The synthesized visible wavelength chromophores will absorb still more strongly in the UV, so without continuing ablation the darkening process is self-limiting. Darkening will occur by transmission of UV radiation into the topmost few optical depths of CH_4 -rich ice.

A thin dark layer rich in organics can be maintained only on a surface that experiences no net accumulation of impact ejecta—because, after a sufficient number of impacts, the ejecta will have been distilled by the impact process, depleting it of CH_4 and leaving it permanently bright. Where net ablation takes place, the surface will be dark, as ice not yet completely depleted in CH_4 is continually exposed to UV irradiation by removal of overlying material and by diffusion of CH_4 up from pristine CH_4 -rich ice close beneath the ablating surface. Where net deposition takes place the surface will be bright, as the accumulating material will have been depleted in CH_4 , and thick accumulations of regolith will hinder upward diffusion of CH_4 .

As described above, the process causing ablation on the leading hemisphere and deposition on the trailing hemisphere is ballistic diffusion, driven by the impact flux gradient. The bright trailing hemisphere will, of course, also have deposited on it organics synthesized on the dark leading hemisphere (perhaps accounting for its reddish colour), but because the zone of organic production is only a few optical depths thick, ejecta from impacts there will be predominantly bright. Organics will thus be present in both hemispheres: on the trailing hemisphere, mixed uniformly through a thick deposited regolith, and on the leading hemisphere, strongly concentrated in a very thin syn-

thesis zone at the surface. Because the albedo of the surface depends on the relative rates of exposure of CH₄-rich darkenable ice and of deposition of CH₄-poor non-darkenable ice, the surface albedo contours should follow the impact flux contours, as observed. The dark-floored crater on the trailing hemisphere may be readily understood if it is a young feature: an impact has recently penetrated the bright regolith, exposing CH₄-rich ice that has since been UV darkened but that has not yet had time to be buried under bright debris.

Our favoured model has implications for several other Solar System objects. Hyperion exhibits a reddish colour and an albedo intermediate between those of the bright and dark materials on Iapetus. It shows no albedo asymmetry. Voyager images suggest that Hyperion is not tidally locked in synchronous rotation²², so no impact flux gradient is expected across its surface. There will, however, be a slow surface ablation due to the low escape velocity. Moderate UV darkening should affect the satellite uniformly, and may account for its observed albedo and colour.

The surfaces of the uranian satellites probably also contain CH₄ and NH₃, so that the darkening process should operate there as well. Because there is no known source of retrograde debris in the uranian system, these satellites should lack the very strong albedo asymmetry of Iapetus, although they may exhibit a less pronounced asymmetry. The available data show Oberon (which lies farthest from Uranus and therefore experiences the shallowest impact flux gradient) to have a darker leading hemisphere and a brighter trailing hemisphere²³. No data exist for the other satellites, and the present pole-on orientation of the uranian system precludes detection of a leading/trailing asymmetry from Earth-based disk-integrated photometry. UV darkening should, as on Hyperion, lead to mean albedos intermediate between those of the leading and trailing hemispheres of Iapetus. Albedos in the range 0.2–0.3 have in fact been reported very recently for the uranian satellites²⁴. Conceivably, UV darkening is also responsible for the very low albedo of the uranian rings, as the very low gravity of the ring particles would prevent regolith accumulation. There will be an opportunity to investigate these possibilities when Voyager 2 encounters Uranus in 1986.

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Electron pitch-angle scattering by low frequency waves at the geomagnetic Equator

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Recent satellite observations¹ have revealed details on the strength of the pulsing extremely low frequency (ELF) hiss in the equatorial region which maps down to the auroral zone. It is shown here to be insufficient to produce pitch-angle scattering of energetic electrons into the loss cone at a sufficiently high rate to explain the characteristics of the measured pitch-angle distributions in pulsating auroras, within the framework of the theory proposed by Coroniti and Kennel². It is suggested that wave-particle interactions involving both broadband incoherent ELF waves and narrow band coherent very low frequency (VLF) waves may be able to produce a sufficiently high rate of pitch-angle scattering.

Pulsating auroras are a common feature of the auroral sub-storm³. Other physical phenomena have been observed to be closely related to the optical pulsations; such as pulsed ELF hiss, X-ray pulsations, cosmic noise absorption (CNA) pulsations and magnetic micropulsations. One theory which attempts to relate some of these phenomena has been put forward by Coroniti and Kennel². They suggest that the presence of magnetic micropulsations near the Equator can modulate the growth rate of whistler mode waves in this region and hence the electron pitch-angle diffusion (PAD) rate. We will now consider the last interaction in this chain, and calculate whether the observed intensity of pulsed ELF hiss is sufficiently strong to give rise to the required rate of pitch-angle diffusion of electrons.

Gough and Korth¹ suggest that data analysed from the GEOS II geostationary satellite gives evidence that equatorial pulsed ELF hiss is directly related to the precipitation of energetic (>22 keV) electrons during pulsating auroras.

Their result arose from analysis of ELF and energetic electron (>22 keV) pitch-angle data from GEOS II for a 14-min period on the 19 November 1979 (22.22–22.36 UT) when visual observations of pulsating auroras over northern Scandinavia were reported (though no actual records were taken). They plot the occurrence frequency of the different ELF hiss intensities for two cases; one where the energetic electron loss cone is partially filled, the other where it is not. Partial filling of the loss cone is arbitrarily defined as being when the minimum electron count rate in the loss cone is >0.4 times the count rate just outside the loss cone. For a partially filled loss cone, they found the most likely ELF intensity to be around 10^{-3} nT Hz^{-1/2} with a 75% probability of being $>10^{-4}$ nT Hz^{-1/2}. For an empty loss cone, the most likely ELF intensity was 10^{-5} nT Hz^{-1/2} with a 75% probability of being $<10^{-4}$ nT Hz^{-1/2}.

In the Coroniti and Kennel theory the electron pitch-angle scattering rate at the Equator is proportional to the strength of the interacting wave noise. Two extremes of PAD are considered: strong diffusion, where an electron is scattered across the loss cone in a time short compared with the quarter bounce period, T_e (that is the time it takes the electron to travel from the Equator down to auroral altitudes); and weak diffusion where electrons diffuse across only a fraction of the loss cone in time, T_e . An observation indicative of strong PAD is that particle fluxes within the loss cone are equal to the trapped particle fluxes (that is, isotropic particle pitch-angle distribution)⁴, while the consequence of weak PAD is an anisotropic particle pitch-angle distribution.

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Several measurements of electron pitch-angle distributions in the energy range 2–80 keV, have been made from sounding rockets flown into pulsating auroras^{5–7}. These clearly show that the electron pitch-angle distributions are isotropic during the pulsation maxima, indicative of strong PAD. It is possible to make an order of magnitude calculation of the minimum wave strength required to produce strong electron PAD and we can compare this value with the wave intensity of the pulsed hiss observed at GEOS (see ref. 8 for details). We will follow Kennel and Petschek⁹ in calculating the diffusion coefficient, D_α , for small, random changes in the pitch-angle, α of the particle in a cyclotron resonance wave-particle interaction, and hence obtain an expression for B'_{SD} , the wave amplitude for strong PAD.

$$D_\alpha \sim \frac{(\Delta\alpha)^2}{2\Delta t} \sim \frac{\Omega_e}{\gamma} \left(\frac{B'}{B}\right)^2 f_{res}$$

where B' is the total wide band amplitude of the fluctuating magnetic field, (Ω_e/γ) is the relativistic electron gyrofrequency, B is the ambient field strength, and f_{res} is the fraction of the electron bounce orbit spent in effective resonance.

For strong diffusion one requires:

$$D_\alpha > D_{SD} = \frac{2\alpha_L^2}{\tau_b}$$

where α_L is the equatorial pitch-angle, and τ_b is the bounce period of particles between mirror points. To calculate a reasonable value for f_{res} , we will assume that the interaction region is a few thousand kilometres in extent, centred on the equatorial plane at $L=6$. A 10-keV electron will traverse this distance in $\sim 4 \times 10^{-2}$ s which can be considered to be the maximum interaction time for half a bounce orbit. At $L=6$, τ_b for a 10-keV electron is ~ 4 s, giving a value of approximately $\frac{1}{50}$ for f_{res} . Taking $\alpha_L^2 = 1/2L^3$ and $f_{res} = \frac{1}{50}$ it is easy to show that the minimum wave amplitude for strong diffusion scattering is:

$$B'_{SD} \sim 7.07 B \left[\frac{\gamma}{\Omega_e \tau_b L^3} \right]^{1/2}$$

Figure 1 shows results at different L values assuming a dipole field and τ_b given by Hamlin *et al.*¹⁰ (note that the GEOS satellite is nominally at an $L \sim 6.5$); the minimum wave amplitude for strong diffusion of 22-keV electrons at this L -value is $\sim 22 \times 10^{-2}$ nT.

Figure 1 (see also ref. 1) shows ELF intensity up to 2×10^{-3} nT Hz^{-1/2} with the highest probability of the waves having this strength when the loss cone is partially filled, which should correspond to the pulsating aurora case. Taking this intensity

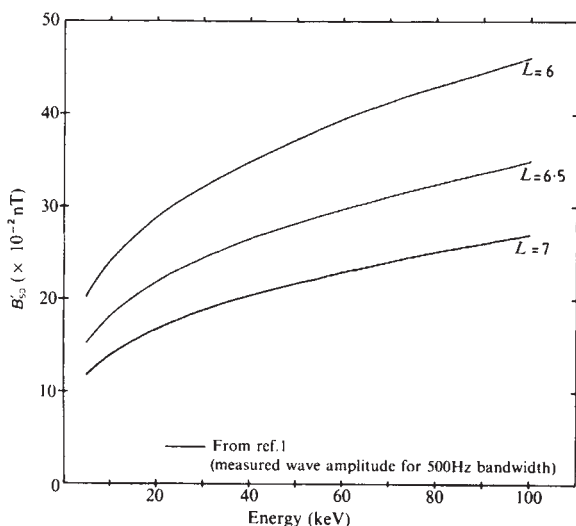


Fig. 1 Variation of minimum wave amplitude required for strong diffusion with electron energy for several L -values.

over a signal bandwidth of 500 Hz we obtain a wave amplitude of $\sim 4 \times 10^{-2}$ nT. In their study of Ogo 5 data, Tsurutani and Smith¹¹ found pulsed ELF hiss with peak intensities of $\sim 3.16 \times 10^{-4}$ nT Hz^{-1/2} and a background intensity of $\sim 10^{-4}$ nT Hz^{-1/2}. Over a bandwidth of 500 Hz, this corresponds to values of B'_{SD} of 7.1×10^{-3} nT and 2.24×10^{-3} nT respectively. Early GEOS data showed even smaller wave amplitudes. At 00MLT (magnetic local time) for strong magnetic activity ($Kp > 4$) a wave amplitude of $\sim 2.1 \times 10^{-4}$ nT Hz^{-1/2} was found for the pulsed ELF hiss. This strength corresponds to $\sim 4.7 \times 10^{-3}$ nT over a 500-Hz bandwidth signal, probably an overestimate of the bandwidth of the ELF signal. In fact, the electrons may only resonate over a very small proportion (~ 100 Hz) of the total ELF signal.

It is clear from this, that the strength of the ELF electromagnetic waves at the Equator is not strong enough to put particles on strong diffusion. It is unlikely therefore, that the equatorial pulsed ELF hiss is wholly responsible for the precipitation of energetic electrons that produce pulsating aurora, within the framework of the Coroniti and Kennel theory. Another explanation must be found for the isotropic electron pitch-angle distributions measured in pulsating auroras.

There is the possibility that an additional process is involved in the pitch-angle scattering of the electrons beside wide-band incoherent whistler mode turbulence. Naturally occurring narrow-band coherent VLF waves have been measured on several satellites¹³, and even moderate amplitude ($\sim 10^{-2}$ nT) waves of this type can produce detectable fluxes of precipitating electrons¹⁴. Wave-particle interactions involving both broadband and narrowband waves may be able to account for the observed pitch-angle scattering rate of electrons, as suggested by Davidson¹⁵.

Note that another piece of information contained in Fig. 1, that is, cases with the partially filled loss cone (pulsating aurora cases), only occur for a fraction of the total time that pulsed ELF hiss is observed, supports previous findings. Isenberg *et al.*¹⁶ found on the SCATHA satellite that while the onset of waves coincided with the onset of enhanced particle intensities, the converse was not true: every instance of chorus activity was not accompanied by particle intensity enhancements. Thomas⁸, using data from an all-sky TV camera operated at the GEOS II footprint position in northern Scandinavia, found that whenever pulsating aurora was seen by the all-sky TV camera, pulsed ELF hiss was seen at GEOS, but the converse was not always true.

The source of pulsating aurora is still under considerable debate (see, for example, refs 17, 18). Isenberg *et al.*¹⁶ found that chorus emissions were associated with clouds of energetic electrons at the geomagnetic Equator. This particle structure may be associated with patches of precipitation in the auroral ionosphere, though the small scale structure in pulsating aurora is likely to be due to local ionospheric structure. In their study of pulsed ELF hiss measured at GEOS and ground-based measurements of pulsating aurora and magnetic micropulsations, Ward *et al.*¹⁹ found several cases where the micropulsations associated with pulsations in the aurora possessed major frequency components which were of ionospheric origin.

Pitch-angle scattering of energetic electrons by whistler mode waves modulated by magnetic micropulsations has been a ubiquitous theory in the literature for explaining details of pulsating aurora over the past decade. However, if the waves involved are of broadband incoherent type, calculations presented here, show that their strength is insufficient to produce the observed amount of pitch-angle scattering in pulsating auroras. It seems likely that a single physical mechanism is unable to explain the sheer variability of pulsating auroras^{3,20,21}, and that a stricter ordering of events is essential to pinpoint the inherent physical mechanisms⁸.

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Factors affecting cricket ball swing

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The basic principles behind cricket ball swing have been understood by scientists for years^{1–3}; however, there has been only one published account of experiments on cricket ball swing⁴. Through a combination of flow visualization and measurements of surface pressures on a cricket ball, the individual parameters responsible for producing swing have been investigated. An accurate correlation between swing, seam angle, spin rate and flow speed was obtained in the more realistic projection tests where spinning cricket balls were projected into a wind tunnel⁵. For a ball to swing well, it should be released at a speed of between 15 and 30 m s⁻¹ with the seam inclined at about 20° incidence and the ball spinning steadily along the seam at about 11 rev s⁻¹. The present experiments do not support the popular view that swing increases in damp or humid conditions. In particular, measurements show that the cricket ball seam does not swell in such conditions.

Fast bowlers in cricket make the ball swing by a judicious use of the seam. The ball is released with the seam at an angle to the airflow. Figure 1 shows a cricket ball held stationary in a wind tunnel with the seam set at an incidence angle of 40° to the airflow. Smoke was injected into the separated region behind the ball where it was entrained right up to the separation points. The boundary layer on the lower surface has been tripped by the seam into turbulence, evidenced by the chaotic nature of the smoke edge just downstream of the separation point. On the upper surface a smooth, clean edge confirms that the separating boundary layer was in a laminar state. The laminar boundary layer on the upper surface has separated relatively early compared with the turbulent boundary layer on the lower surface. This asymmetry is further confirmed by the upwards deflection of the wake flow behind the ball.

To investigate the surface pressures on a cricket ball, a normally manufactured ball was furnished with 24, 1-mm pressure tappings along its equator, the core having been removed. Figure 2 shows the measured pressures on the ball mounted in a wind tunnel with the seam set at an incidence of 20°.

At low flowspeeds, the pressure distributions on the two hemispheres are equal and symmetrical, so there would be no side force. At $U = 25 \text{ m s}^{-1}$ the pressure dip on the right-hand face of the ball is clearly lower than that on the left-hand face. This would result in the ball swinging towards the right. The maximum pressure difference between the two sides occurs at $U = 29 \text{ m s}^{-1}$ when, presumably, the boundary layer on the

Table 1 Parameters of balls under test

Ball No.	Pieces in cover	Seam stitching type	No. of stitches per row
8	4	Hand-sewn	60
11	4	Hand-sewn	60
14	4	Hand-sewn	80
17	2	Machine-sewn	60
21	4	Machine-sewn	60

seam side (right-hand side) is fully turbulent while that on the non-seam side is still laminar. Even at the highest flow speed achieved in this test ($U = 36.5 \text{ m s}^{-1}$), the asymmetry in pressure distributions is still clearly indicated although the pressure difference is reduced. The actual (critical) flow speeds at which the asymmetry appears or disappears was found to be a function of the seam angle, surface roughness and free-stream turbulence—in practice it would also depend on the spin rate of the ball.

When a cricket ball is bowled, there will always be some backspin imparted to it which helps to stabilize the seam orientation. In this series of experiments, forces were measured on spinning cricket balls projected into a $0.91 \times 0.61 \text{ m}$ wind tunnel. This wind tunnel has a maximum speed of $\sim 40 \text{ m s}^{-1}$ with a turbulence level of $\sim 0.2\%$. The ball was rolled along the seam down a 1 m ramp located on the working section roof and into the airflow through a slot. The forces due to the wind can then be evaluated once the landing point, and hence the deflection from the datum, has been measured. The spin rate was varied by changing the starting point along the ramp and the seam angle was varied by adjusting the alignment of the ramp with the airflow. Typically, the ball took $\sim 1/3 \text{ s}$ to fall through the working section resulting in a maximum deflection of $\sim 200 \text{ mm}$. In all, 23 balls were tested. Five balls (one from each quality type) were tested extensively (Table 1).

Figure 3 shows the averaged results in the form of side force, normalized by the weight of the ball, plotted against flow speed. At nominally zero seam angle there is no significant side force, except at high speeds when the local roughness can start to have effect. However, when the seam is set at an incidence to the oncoming flow, the side force is seen to increase with flow speed up to a maximum of ~ 0.3 before falling off rapidly. The critical flow speed at which the side force starts to fall is $\sim 30 \text{ m s}^{-1}$, which corresponds to a Reynolds number of $\sim 1.5 \times 10^5$.

Of the five balls tested extensively, ball 17 was found to swing the most, followed by balls 11, 14, 8 and 21. Ball 17 was the only two-piece ball and this result was therefore not too surprising. The secondary seam on a four-piece ball produces

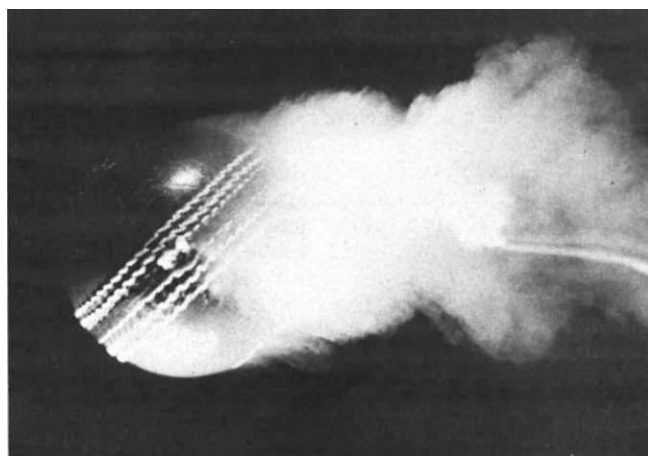


Fig. 1 Smoke photograph of flow over a cricket ball. Flow is from left to right. Seam angle, 40°; flow speed, 17 m s^{-1} ; Reynolds number, 0.85×10^5 .

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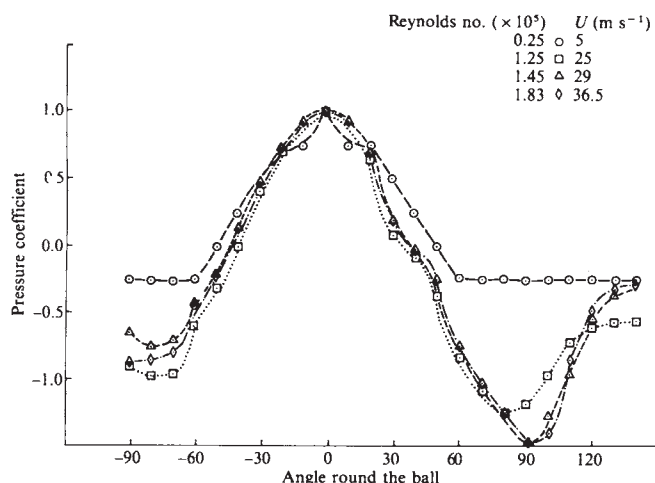


Fig. 2 Pressure distributions on a cricket ball held at an incidence of 20° .

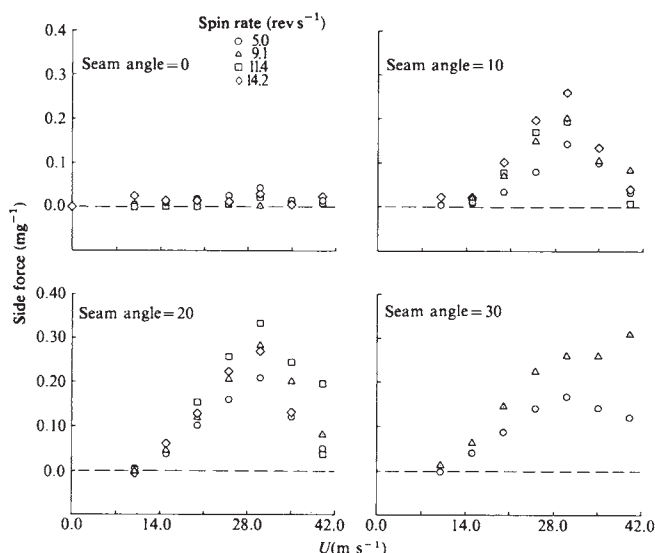


Fig. 3 Side-force variation averaged over balls 8, 11, 14, 17 and 21—normalized by the weight of the ball.

an effective roughness when the ball is spinning and this causes early transition of the laminar boundary layer on the non-seam side. On inspecting the primary seam on these balls it was apparent that the hand-sewn seam was more prominent than the machine-sewn seam. Also, the seam on ball 14 with 80 stitches felt distinctly rougher than the seam with only 60 stitches. However, in the swing rankings ball 17, with a machine-sewn seam of 60 stitches came first. Thus, it seems that the primary seam on a new ball, regardless of its stitching type and number of stitches, is enough to trip the boundary layer over the speed range of interest.

It is clear from the averaged results (Fig. 3) that at a bowling speed of $\sim 30 \text{ m s}^{-1}$ (70 m.p.h.), maximum swing is obtained at a seam angle of 20° and a spin rate of 11.4 rev s^{-1} . However, at lower speeds ($< 20 \text{ m s}^{-1}$) and at lower seam angles ($\sim 10^\circ$), it is better to have a higher spin rate of about 14 rev s^{-1} . Incidentally, this is comparable to the highest spin rate measured on fast bowlers. At low bowling speeds it is also better to have a higher seam angle of $\sim 30^\circ$.

Spin on the ball helps to stabilize the seam orientation. Basically, for stability, the torque due to spin should be greater than the torque about the vertical axis due to the flow asymmetry. Too much spin can be detrimental as the effective roughness on the ball's surface is increased (that is, the critical Reynolds number is reached sooner). Note that in practice it is not easy to control the amount of spin imparted to the ball.

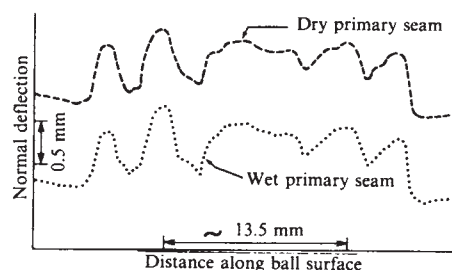


Fig. 4 Talysurf contour plots of the primary seam to investigate effects of humidity.

As for seam angle, it is better not to trip the boundary layer too early (low seam angle) since a turbulent boundary layer grows faster than a laminar boundary layer and a thicker layer will separate early. At the same time, the seam angle should not be so large that the boundary layer becomes turbulent before reaching the seam. The effect of the seam on a turbulent boundary layer is to make it thicker, thus driving it closer to separation. So, at bowling speeds of $20\text{--}30 \text{ m s}^{-1}$, a seam angle of $\sim 20^\circ$ turns out to be optimum. However, at lower speeds, especially precritical ($< 15 \text{ m s}^{-1}$) the seam angle needs to be larger ($\sim 30^\circ$) so that by the time the flow accelerates round to the seam position, the critical velocity for tripping has been reached. The results for the optimum seam angle are consistent with those obtained by Barton⁴. However, his optimum spin rate is about half that suggested by the present experiments. This may be at least partly attributed to the relatively high turbulence levels in his wind tunnel ($\sim 1\%$).

It is widely believed that humid or damp days are conducive to swing bowling, but there has been no scientific proof of this. The changes in temperature and pressure normally encountered are insufficient to change the air density and viscosity, and hence Reynolds number, significantly. The most popular explanation is that the seam swells by absorbing water vapour thus making it a more efficient boundary layer trip. A series of experiments were performed to investigate this possibility.

A very low pressure rotary talysurf with a sensitivity of 40:1 was used to obtain surface contour plots. Figure 4 shows talysurf plots for a traverse across the primary seam on a new ball before and a few minutes after soaking in water. Even in this extreme case, there is no sign of any change in the seam dimensions. The same test on a used ball also proved negative.

A more controlled test was also performed in which two balls were placed in a humidity chamber with a relative humidity of 75% for 48 h. Photographic tests showed no real effect on the seam or the ball surface. The projection test on these two balls with the surface dry, humid and wet also indicated no increase in side force.

It is possible that dampness affects the amount of spin imparted to the ball. We found that the varnish painted on new balls reacts with moisture to produce a rather tacky surface. A new ball with dry varnish is normally very slippery so the tacky surface would result in a better grip and hence more spin, and as shown above, an increase in spin rate (of at least up to 11 rev s^{-1}) certainly increases the side force. So perhaps without actually realizing it, the bowler just imparts more spin on a damp or humid day.

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Lignin depolymerizes coal at 300 °C

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Liquefaction of coal requires thermal rupture of its carbon-carbon bonds to form free-radical fragments. Before these radicals can recombine or polymerize they must be stabilized by hydrogen atoms usually supplied by a hydrogen-donating solvent or by gaseous hydrogen. The conditions required for such liquefaction reactions are usually temperatures ≥ 400 °C and hydrogen pressures of several hundred atmospheres^{1,2}. Often catalysts can be used to accelerate the breaking of carbon-carbon bonds and the transfer of hydrogen. Ouchi *et al.*⁵ reported that *p*-toluenesulphonic acid catalysed the depolymerization of coal. Darlage and Bailey⁶ investigated various solvents and Friedel-Crafts catalysts and found that repolymerization of the coal radical fragments was a predominant reaction when coal liquefaction was conducted in non-phenolic solvents such as toluene, xylene or tetralin. Heating coals in phenol at 425 °C was reported⁷ to cause extensive depolymerization accompanied by rearrangement of hydrogen to form a hydrogen-rich soluble fraction and a hydrogen-poor insoluble fraction. We now report that bituminous coal can be depolymerized at 300 °C by using, instead of phenol, the abundant, renewable raw material lignin which is a major component of all vascular plants. When a mixture of coal and lignin is reacted in a solvent in the presence of an acid catalyst both the coal and the lignin depolymerize in relatively mild conditions to form a filterable liquid product. The presence of the lignin increases substantially the amount of the coal that can be so liquefied.

Measured amounts of predried coal (Illinois no. 6, 74–105 μm) and lignin (alkali extracted from steam-exploded Aspen) were reacted in tetralin with a solid catalyst at 300 °C and pressures of ~ 100 –130 atmospheres for 3 h. After reaction, the mixture was paper-filtered and the liquid was vacuum-distilled into several fractions. Each fraction was analysed by gas chromatography-mass spectrometry (GC-MS).

The overall conversion Y (%) of solids to liquid products was computed from the expression:

$$Y = \frac{M_1 - M_2}{M_1} \times 100$$

where M_1 is the mass of lignin + coal (maf basis) charged to the reactor and M_2 is the mass of solid residue (less any solid catalyst added and less the ash in the coal) after the reaction. Table 1 summarizes the various experiments, the reaction conditions and the overall conversion obtained. Several experiments (1-E to 1-I) were conducted with lignin but with no coal in the reaction mixture using tetralin/lignin ratios ranging from 4 to 8 ml g⁻¹. In these experiments the range of conversions of lignin to filterable liquid was 30–38% with a mean of 34%. For the other experiments, in which coal was also present in addition to the lignin, it was assumed that the conversion of lignin remained at 34%. With this assumption of constant lignin conversion it was possible to estimate the conversion of coal to liquid products, X (%), by the expression:

$$X = \frac{(Y/100)(M_L + M_C) - 0.34M_L}{M_C} \times 100$$

where M_L and M_C are the initial masses of lignin and coal respectively.

In this way the overall conversion of the mixed coal plus lignin reactants was used to estimate the conversion of coal by

Table 1 Reaction conditions and overall conversions for experiments at high pressure*. Coal—Illinois no. 6 (74–105 μm), reaction time of 3 h and reaction temperature of 300 °C

Experiment	Lignin mass (g)	Coal mass (g)	Solvent tetralin volume (ml)	Catalyst mass (5.0 g)	Reaction pressure (p.s.i.)	Overall conversion Y (%)	Estimated coal conversion X (%)
1-A	0.0	50	200	SiO ₂ -Al ₂ O ₃	1,700	5.2	5.2
1-B	10	40	200	SiO ₂ -Al ₂ O ₃	1,550	22.4	19.1
1-C	15	35	200	SiO ₂ -Al ₂ O ₃	1,450	28.7	26.1
1-D	25	25	200	SiO ₂ -Al ₂ O ₃	1,700	34.3	34.7
1-E	25	0.0	200	SiO ₂ -Al ₂ O ₃	1,500	35.0	—
1-F	25	0.0	200	SiO ₂ -Al ₂ O ₃	1,600	30.0	—
1-G	50	0.0	200	SiO ₂ -Al ₂ O ₃	1,600	31.0	—
1-H	50	0.0	200	SiO ₂ -Al ₂ O ₃	1,650	36.0	—
1-I	50	0.0	200	SiO ₂ -Al ₂ O ₃	1,650	38.0	—
1-J	35	15	200	SiO ₂ -Al ₂ O ₃	1,400	36.5	43.3
1-K§	0.0	50	150	NiO-MoO ₃ on Al ₂ O ₃	1,650	0.0	0.0
1-L	50	0.0	200	NiO-MoO ₃ on Al ₂ O ₃	1,800	37.0	—
1-M	20	0.0	450	—	1,700	63.0	—
1-N¶	0.0	20	450	—	1,700	3.0	3.0
Filtrate of 1-M							
1-O	50	0.0	200	NiO-MoO ₃ §§	1,800	38.5	—
1-P#	25	25	200	NiO-MoO ₃ on Al ₂ O ₃	2,350	83.0	66
1-Q#	25	25	200	—	2,350	80.0	60
1-R**	25	25	150	NiO-MoO ₃	1,600	34.8	—
1-S††	25	25	100	NiO-MoO ₃	1,900	52.8	5
1-T‡‡	25	25	0.0	NiO-MoO ₃	1,500	80.0	60

* In each experiment the initial pressure was 1,000 psi (pounds per square inch) applied from a H₂ cylinder. Final pressures ranged from 1,400–2,350 psi. The products from experiments 1-C, 1-D, 1-J and 1-L were identified by GC-MS. See Table 2.

† Mass of coal containing 13.5% ash.

‡ 25% of glass spheres was added to the reaction mixture for experiment 1-F.

§ 50 ml of the liquid reaction product from experiment 1-L was included in the reaction mixture of experiment 1-K.

|| 20 g of phenol was added to the reaction mixture of experiment 1-M.

¶ 450 ml of the filtrate from experiment 1-M was used as the solvent for experiment 1-N.

These two experiments were carried out at 400 °C for only 1 h.

** 50 ml quaiacol was added to the 150 ml tetralin, total volume was 200 ml.

†† 100 ml quaiacol was added to 100 ml of tetralin, total volume was 200 ml.

‡‡ 200 ml quaiacol was the only solvent used in this experiment.

§§ NiO-MoO₃ catalyst gives slightly higher liquefaction yield.

a correction based on the assumption that 34% of the original lignin was always converted to liquid products.

The conversions of coal so estimated are summarized in Table 1. It is evident that the presence of lignin in the reaction mixture caused considerable depolymerization and liquefaction of the coal, with conversions to filterable liquids ranging from about 19.1 to 43.2%. In comparable conditions, but with no lignin present, only about 5.2% of the coal was converted (experiment 1-A).

The experiments summarized in Table 1 show in general that $\text{SiO}_2\text{-Al}_2\text{O}_3$ or NiO-MoO catalyse lignin depolymerization but not coal liquefaction at 300 °C. They also show that the presence of lignin in the reaction mixture enhances coal liquefaction at this low temperature. Experiment 1-K shows, however, that pre-reacted lignin (from experiment 1-L) is not effective in enhancing coal liquefaction; this stands in contrast to the significant liquefaction caused by phenol (experiment 1-M). It appears, therefore, that the favourable effect of lignin in liquefying coal may be attributed to the active molecular fragments formed by thermolysis of the lignin within the reaction mixture.

The most extensive coal liquefaction was obtained in experiments 1-P and 1-Q conducted at a higher temperature (400 °C) but for only 1 h. Experiment 1-Q suggests that a solid catalyst may not be necessary for coal liquefaction in the presence of lignin and tetralin at 400 °C.

Addition of a supplementary source of radicals, such as quaiacol (necessary to initiate the radical chain reactions) increased the conversion of the reactions (1-R through 1-T) from 35 to 80%, for 50 and 200 ml of quaiacol, respectively.

Table 2 lists the major molecular species detected by GC-MS in the liquid products from some selected experiments. Three-ring aromatic structures were detected only when coal was used, as expected.

In experiments 1-R to 1-T progressively larger amounts of guaiacol added to the reaction mixture are seen to produce correspondingly larger conversions of lignin and coal. Guaiacol is believed to provide additional radicals to initiate chain reactions important in the liquefaction process.

The catalytic effect of lignin on depolymerization of coal at 300 °C is related to the hydrocracking of lignin observed by many¹⁰⁻¹³ at comparable temperatures which are lower than temperatures normally required for coal liquefaction (400–500 °C). Early work in Japan⁹ and in the US by Goheen^{10,11} demonstrated that distillable phenols could be produced from lignin in yields up to 22%; later workers¹² reported yields as high as 38–42%.

Thermal and hydrolquefaction of coal and of lignin is believed to proceed by a free-radical mechanism^{1-4,8,13,15} in

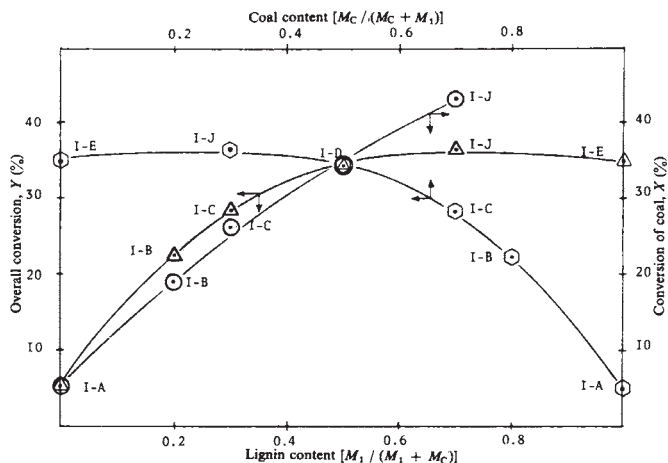


Fig. 1 Influence of lignin content of reaction mixture on overall conversion and coal conversion. Also influence of coal on overall conversion. Each data point is labelled with the corresponding experiment identification in Table 1. ○, Conversion of coal to liquid versus lignin content of reaction mixture. △, Influence of lignin content of reaction mixture on overall conversion of solids to liquid. ○, Influence of coal content of reaction mixture on overall conversion of solids to liquid.

which the primary step is thermal, homolytic bond rupture to form radical fragments. For the experiments reported here, we hypothesize that thermal degradation of lignin produces substituted phenoxyl radicals (as well as other types) at temperatures (300 °C) too low for substantial thermolysis of the coal molecules. The inherent resonance stabilization of the phenoxyl radicals insure they exist sufficiently long to encounter and react with coal molecules to cause depolymerization. The O-R bonds are among the weakest (60 kcal mol⁻¹) in lignin and should rupture thermally to produce phenoxy radicals from alkylphenylethers.

In a typical bituminous coal, aromatic and hydro-aromatic clusters containing two to three rings per cluster are joined by methene linkages one to three carbon atoms in length. On the other hand, lignin contains repeating structural units related to phenylpropane; in the lignin polymer, monoaromatic rings are linked by various C-C and C-O bonds. The ether bonds linking the monoaromatic nuclei in lignin have dissociation energies of about 60–75 kcal mol⁻¹, whereas the energies for the more stable methene bridges in coal fall in the range of 100–110 kcal mol⁻¹. It seems reasonable that the substituted phenoxyl radicals formed by thermolysis of lignin could react to cleave the methene bridges of coal, thereby forming the anthracene, naphthalene and phenanthrene-type products observed (Table 2). The first step in the C-C bond breaking process is probably hydrogen atom abstraction from methene (C-H bond energy of about 86 kcal mol⁻¹) by phenoxy radicals to form phenol (O-H bond energy of ~100 kcal mol⁻¹).

Addition of guaiacol, as a supplementary source of phenoxy radicals enhanced the rate of liquefaction and increased the liquid yields to 80% in comparable conditions. This result supports the radical chain mechanism proposed here for coal liquefaction at low temperature in the presence of lignin.

No coal liquefaction was observed when previously hydrocracked lignin was used. Presumably the phenoxy radicals in this preparation had been deactivated by absorbing hydrogen. When lignin was directly mixed with coal, however, it formed radicals *in situ* which could then react immediately with coal molecules. Some additional coal liquefaction might also be attributed to dissolution in the better solvent provided by polar phenoxy products, formed in the reaction.

At lower lignin concentrations in the reaction mixture the overall liquefaction of (coal+lignin) increases monotonically with added lignin until about equal masses of lignin and coal are present. Further increase in the proportion of lignin has

Table 2 Compounds identified by GC-MS in the products from selected experiments

Expt	Major components in reaction products
1-C	
1-D	
1-G	
1-J	
1-L	

little effect on overall conversion. This levelling-off behaviour which is shown in Fig. 1 may be attributed to recombination of lignin radicals with each other at the higher lignin concentrations. On the other hand, Fig. 1 also shows that the liquefaction of coal continues to rise with lignin concentration in the reaction mixture over the entire range of lignin content studied.

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Carbon isotopic variation in spectral type II diamonds

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The carbon isotope composition of most diamonds measured so far¹ falls within a fairly restricted range from -5 to -9%. On the other hand, the type II diamonds studied here show $\delta^{13}\text{C}$ values (-0.5 to -31.9%) extending over almost the entire range of carbon isotope values previously quoted for diamonds. We suggest here that these data are related to variations in the nature of the carbon reservoir source and associated inclusion suites.

One of the fundamental enigmas of terrestrial carbon isotope geochemistry is that diamonds exhibit a carbon isotope range of 30‰ yet are believed to have a deep crustal or mantle origin and may be samples of 'primordial' or non-recycled carbon. The database for carbon isotopes in diamonds is derived from numerous publications with the notable contributions from Deines¹ and Kovalskii and Cherskii². Most such measurements yield $\delta^{13}\text{C}$ values of between -5 and -9‰ (ref. 1) and have thus given rise to the premise that the $\delta^{13}\text{C}$ of the Earth's mantle has approximately this composition. More difficult to explain are values extending from +2.7 to -34.4‰. In the past, such data have been regarded as representing unusual cases and the most frequent explanation is that they reflect the participation of the sedimentary carbon cycle in the formation of such diamonds².

An understanding of the variability of carbon isotopes is made more difficult by the fact that most analyses have been

Table 1 $\delta^{13}\text{C}$ analyses of spectral type II diamonds

Description	Run no.	$\delta^{13}\text{C}_{\text{PDB}}$ (‰)
Single fragment IIA	1914	-14.79
	1910	-4.29
	1813	-17.23
	1828	-0.48
	1833	-16.07
	1641	-31.25
	2054	-24.54
	2056	-31.30
Fractured fragment IIA	5692	-23.80
	5690	-31.60
	5704	-31.88
	5708	-31.62
	5710	-31.43
	5698	-31.10
	5700	-31.61
Powdered mixture IIA	821	-17.17
	839	-17.75
	842	-18.02
	*	-17.74
	*	-17.50
	*	-17.39
Powdered fragment IIA	*	-21.08
	*	-21.10
Fractured fragment IIB	5684	-18.73
	5686	-18.57
	5688	-19.01
Crushed fragment IIB	5658	-14.56
	5656	-14.55
	4608	-20.84
	4610	-20.81
	4606	-20.55

Bracketed values are replicates.

* Analysed at the Institute of Geological Sciences.

undertaken on diamonds which may have been heterogeneous on the basis of UV and optical spectra, cathodoluminescence, X-ray diffraction patterns, impurity content and etching behaviour and which are, for the most part, uncharacterized as regards their type. Diamonds are now classified according to their IR spectra³ and electron spin resonance⁴ which depend on the presence and form of nitrogen within the samples⁵. Four main categories are recognized; most natural diamonds belong to type IA. Type IIA diamonds constitute a small percentage, and those from the Premier Mine show some evidence of a bimodal size distribution⁶. Natural specimens of the remaining categories, IB and IIB, the latter known almost exclusively from the Premier Mine, are extremely rare. A compilation of all the available diamond carbon isotopic analyses by Deines¹ suggests a nonrandom distribution with peaks at $\delta^{13}\text{C}$ values other than the mode in the vicinity of -5 to -6‰ and it is therefore possible that all four well-defined diamond types exhibit significant isotopic differences. Since type IA constitutes ~99% of all diamonds, a single specimen with $\delta^{13}\text{C} = -18.6\%$ found among a random 112 Premier Mine diamonds which had $\delta^{13}\text{C}$ values in the range -10 to -2‰¹ is in about the right proportions for the common type of diamond to have the common isotope ratio whereas rarer specimens could be more variable.

We have undertaken a survey of type II diamonds to ascertain whether there is any systematic association between $\delta^{13}\text{C}$ and diamond type, using the sealed tube combustion technique⁷; data are reported relative to PDB.

The results shown in Table 1 are for a range of type II diamonds. All the individual samples have been authenticated as type II by the IR method; IIA being characterized by the absence of absorption bands in the range 7-9.5 μm wavelength

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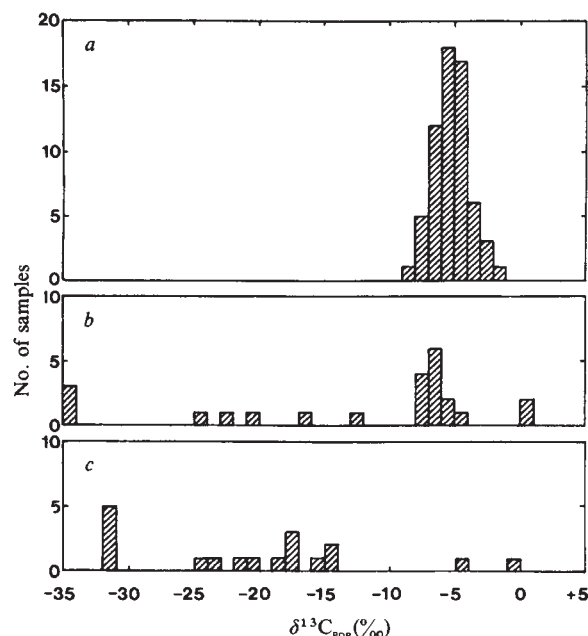


Fig. 1 Distribution of carbon isotope measurements with diamond type. *a*, Containing ultramafic inclusions; *b*, containing eclogite inclusions; *c*, having type II spectral absorption. Data for *a* and *b* from Sobolev *et al.*¹⁰.

whilst IIB were selected on the basis of an absorption at 3.6 μm . First, the most remarkable observation from the data in Table 1 is the wide range of the isotopic compositions which have been encountered. The spread of $\delta^{13}\text{C}$ values from -31.9 to -0.5% covers almost the entire scale previously reported for diamonds. The powdered mixture of IIA samples which can be considered as an average of more than 30 stones falls about the mid-point of the observed range. By comparison, a series of type IA stones used for single diamond homogeneity studies⁷, despite their internal variability, all gave $\delta^{13}\text{C}$ values between -11.0 and -6.4% as did three other type IA stones (-8.6 , -9.1 and -10.7%) analysed in a bulk fashion as part of the current survey. Perhaps significantly, only one of our type IIA diamonds has a $\delta^{13}\text{C}$ value within or close to the commonly observed range identified by the Deines¹ compilation of available data. Second, although no systematic study of the internal consistency of type II stones was attempted, it should be noted that random replicate samples from fractured single diamonds show $\delta^{13}\text{C}$ values which vary by 0.5% or less whereas in coated specimens a spread of up to 4% was encountered⁷.

The above results confirm that diamonds of type IIA and IIB are, on average, isotopically lighter than type IA, but with a spread of $\delta^{13}\text{C}$ values which covers the entire gamut of diamond isotope compositions. Because previous workers have not recorded IR spectra for their specimens, it is not known which of the light $\delta^{13}\text{C}$ values in the literature correspond to type II samples. It is also too early to say whether any distinction between the type IIs exists; only a much larger data set can resolve this problem. The very rare type IB ought also to be studied for the sake of completeness.

The important question is how can differences in origin and provenance account for the observed isotopic composition of type II diamonds? We have already suggested⁷ that an apparent regular pattern of increasing ^{13}C abundance in coated stones is indicative of a fractionation process possibly according to Rayleigh distillation. This fractionation and the relative constancy of the carbon isotopic signature of type IA seems to indicate that the commonest of diamonds were produced in a closed system. In contrast, however, type II diamonds are possibly sampling an open reservoir where fractionation is minimal, but whose variable carbon isotopic composition is

dictated largely by the nature of recycled crustal material. In this context, it should be recalled that the compilation of all diamond $\delta^{13}\text{C}$ analyses does not show a regular distribution¹; the observed pattern might reflect the distinctive isotope signatures of various subducted sedimentary inputs in marine carbonate, marine and terrestrial sedimentary organic carbon.

Previous attempts to categorize the carbon isotope composition of diamonds have met with only limited success. Kovalskii and Cherskii² claimed that the wider $\delta^{13}\text{C}$ range was applicable only to coloured crystals, but Deines¹ demonstrated that for samples from the Premier and Dan Carl mines, South Africa, isotope variations attributable to colour and crystal habit were $<1\%$.

Meyer⁸ and Meyer and Boyd⁹ showed that mineral inclusions in diamond could be assigned to two suites—ultramafic (peridotitic) and eclogitic. This division has been confirmed by several workers, but the observations of greatest relevance in the present context are those of Sobolev *et al.*¹⁰ who have made the carbon isotope measurements on 92 diamonds from which inclusions were extracted and analysed. Their results show that whilst diamonds containing ultramafic inclusions usually have $\delta^{13}\text{C}$ values in the common -9 to -2% range (Fig. 1*a*), about 40% (10 out of 23 samples) from the much rarer eclogitic suite gave anomalous, both light and heavy, isotopic compositions (Fig. 1*b*). The spread of $\delta^{13}\text{C}$ values for the eclogitic suite was -34.4 to $+0.5\%$, almost identical to that observed for the type II diamonds during the current study (Fig. 1*c*).

Given the above apparent correlation, it is tempting to suggest that the eclogitic suite and spectral type II are synonymous. A problem which exists with this hypothesis is that although inclusions from different suites have never been observed to coexist in the same diamond, diamonds from both suites are sometimes found in the same source. Similarly, UV absorption and etching resistance suggest that type I and II material seem to occur in close proximity. The latter difficulty might be explained if diamond seeds from one formation regime may form the basis of crystal growths in another, but there are many circumstances in which type I and II areas are so intimately interspersed that it is scarcely possible to envisage separate origins.

However, situations where diamonds seem to contain material of mixed spectral type could in fact be errors of classification. Most identifications will have been made using UV spectral typing which is now known to be fallible in terms of its ability to recognize type II specimens¹¹.

Aluminium is used in diamond synthesis experiments to getter nitrogen¹²; the presence of aluminium in inclusions such as garnet and diopside could lead to natural gettering and explain the existence of small regions of UV transparent, etch resistant, non-cathodoluminescent (all classical indicators of type II) material in type I specimens¹³. More information relating to examples of mixed spectral type should be obtainable by adopting the laser dissection approach described in an accompanying paper⁷.

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Carbon isotopic variation within individual diamonds

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Various regions of type I diamonds have been analysed to determine intra-specimen variation in carbon isotopic abundance. We report here that generally, although not in all cases, a trend was observed from the centre to the exterior. The cores of some samples were isotopically light (enriched in ^{12}C) whereas the edges became progressively heavier. For the specimen showing the greatest spread of $\delta^{13}\text{C}$ values, the range observed covered almost 4‰ from -11.01 to -7.32 ‰. Such changes could be interpreted according to one or a combination of fractionation processes. Since diamonds exhibit a wide range of $\delta^{13}\text{C}$ values ($+2.7$ to -34.4 ‰)^{1,2} and many specimens show evidence of heterogeneity, we have looked for isotopic differences between areas in individual diamonds which might reasonably be expected to have been formed in different conditions. The small scale of such internal structures in diamond requires the accurate excision of selected volumes. Such a requirement can now be met by controlled laser dissection. As a preliminary to realizing the full potential of such an approach, we have examined the exteriors and cores of coated diamonds, as representing one of the most conspicuous instances in which two distinct epochs of diamond genesis might be involved.

A {100} section sawn from slightly off-centre of a type I coated stone (original weight ~ 0.5 g) and polished to a thickness of 0.4 mm was laser sectioned into four zones as shown in Fig. 1. Any graphite that could have been residual from the cutting operation was removed by sodium nitrite fusion. The different areas of the stone were all shown by means of their IR absorption spectra to be type I although the transparent cores showed 7.3- μm absorption band and thus contained platelet nitrogen whereas the opaque coat did not³. The fragments labelled 1–4 (Fig. 1) of each concentric section were fractured and single pieces of about 100 μg each were taken for analysis.

Carbon isotope measurements were performed on CO_2 produced from the sample by stepwise combustion and sealed-tube techniques according to the methods described elsewhere⁴. These extraction methods exhibit system blanks of <0.1 μg , $\sim 0.1\%$ of the sample size. The CO_2 produced was purified according to conventional procedures and measured using a VG-Micromass 602E to a precision of ± 0.05 ‰. Results were corrected and reported relative to PDB⁵. All the samples analysed gave yields of CO_2 within acceptable error limits ($\pm 5\%$) bearing in mind the difficulties of accurately weighing microgram samples.

Data for 10 individual analyses are listed in Table 1; $\delta^{13}\text{C}$ values spread over a range of almost 4‰ from -7.32 to -11.01 ‰ with the heaviest isotopic compositions being found at the rim and the lightest in the centre. This spread may be placed in perspective when it is considered that 12 samples of standard graphite combusted by similar methods over a period of 6 months gave a $\delta^{13}\text{C}$ value of 28.72 ± 0.21 ‰ (1σ). Averaging the data from the various regions of the diamond also shows a trend of increasing ^{13}C abundance from the interior to the exterior. There is no reason to believe that the heterogeneity encountered is an artefact of the experiment. Samples which were analysed in a stepwise fashion did not show any marked change in isotopic composition between a first and second

Table 1 $\delta^{13}\text{C}$ analyses of selected samples from various regions of a 6-mm cross-section coated diamond

Fraction	Location	Replicate $\delta^{13}\text{C}$ values (‰)	Mean $\delta^{13}\text{C}$ (‰)
1	Core	$-9.23, -11.03, -9.78$	-10.01
2	Outer core	$-9.37, -8.94, -8.36$	-9.16
3	Inner coat	$-8.57, -7.54$	-8.06
4	Coat	$-7.32, -8.28$	-7.80

extraction therefore, the variation seen between samples in Table 1 cannot be attributed to kinetic isotopic fractionation brought about by incomplete reaction. Similarly all the isotopic data in Table 1 are for carbon which was liberated above 600 °C, therefore, the existence of variable amounts of low-combustion temperature contaminants⁶ cannot be proposed to explain the observed fluctuations.

Although the exact location of the chips taken for analysis is unknown within a given rectangle, this is not important because rectangular sections themselves correspond to growth zones. Therefore, variations within any rectangle probably indicate random fluctuations superimposed on an underlying trend. Two pieces of information favour this hypothesis.

First, in addition to the diamond discussed above, five specimens have been investigated in less detail to ascertain whether the phenomenon of variable isotopic composition was commonplace or unusual. All samples were combusted in a single step. The $\delta^{13}\text{C}$ data obtained are shown in Table 2. These samples seem to display a general tendency for the centre of the diamond to be enriched in ^{12}C relative to the periphery on a scale which is significant in view of the small size of the specimens but there are still $\delta^{13}\text{C}$ variations within the regions.

Second, studies by Gurkina *et al.*⁷ on three morphologically distinct diamond crystals (octahedron, octahedron/rhombic dodecahedron and cube) showed a range of isotopic compositions (up to 0.5‰) which, although much smaller in scale than the 4‰ encountered here, follow the same trend in going from lighter at the centre to heavier at the edge.

In considering how 4‰ variations in isotopic composition might arise, some of the more important characteristics of coated stones should be borne in mind; it is clear that conditions did change in some way during their formation. Thus, it has been recognized that the interior or core is generally transparent and frequently octahedral in shape, whereas the outer layer or coat is usually opaque, contains appreciable amounts of chemical impurities such as Fe and Si and often has large numbers of small cavities. Coated stones are seldom perfect octahedra and usually exhibit {110} and {100} forms in addition

Table 2 $\delta^{13}\text{C}$ analyses of selected regions from five coated diamonds

Sample	Location	Replicate $\delta^{13}\text{C}$ values (‰)
A	Core	$-7.29, -7.18, -8.21$
	Interior	-7.19
	Coat	$-6.80, -6.36, -6.99$
B	Core	$-7.35, -7.16$
	Interior	-8.20
	Coat	$-6.70, -8.21$
C	Core	-8.82
	Coat	-7.16
D	Core	$-8.81, -9.07, -7.68$
	Outer coat	$-8.50, -7.70$
	Inner coat	$-8.11, -7.42, -7.84$
	Coat	$-7.45, -7.30$
E	Core	$-9.22, -8.82, -8.50$
	Outer core	$-8.90, -7.60, -7.10$
	Inner coat	$-7.23, -6.90, -7.60$
	Coat	$-7.23, -9.45$

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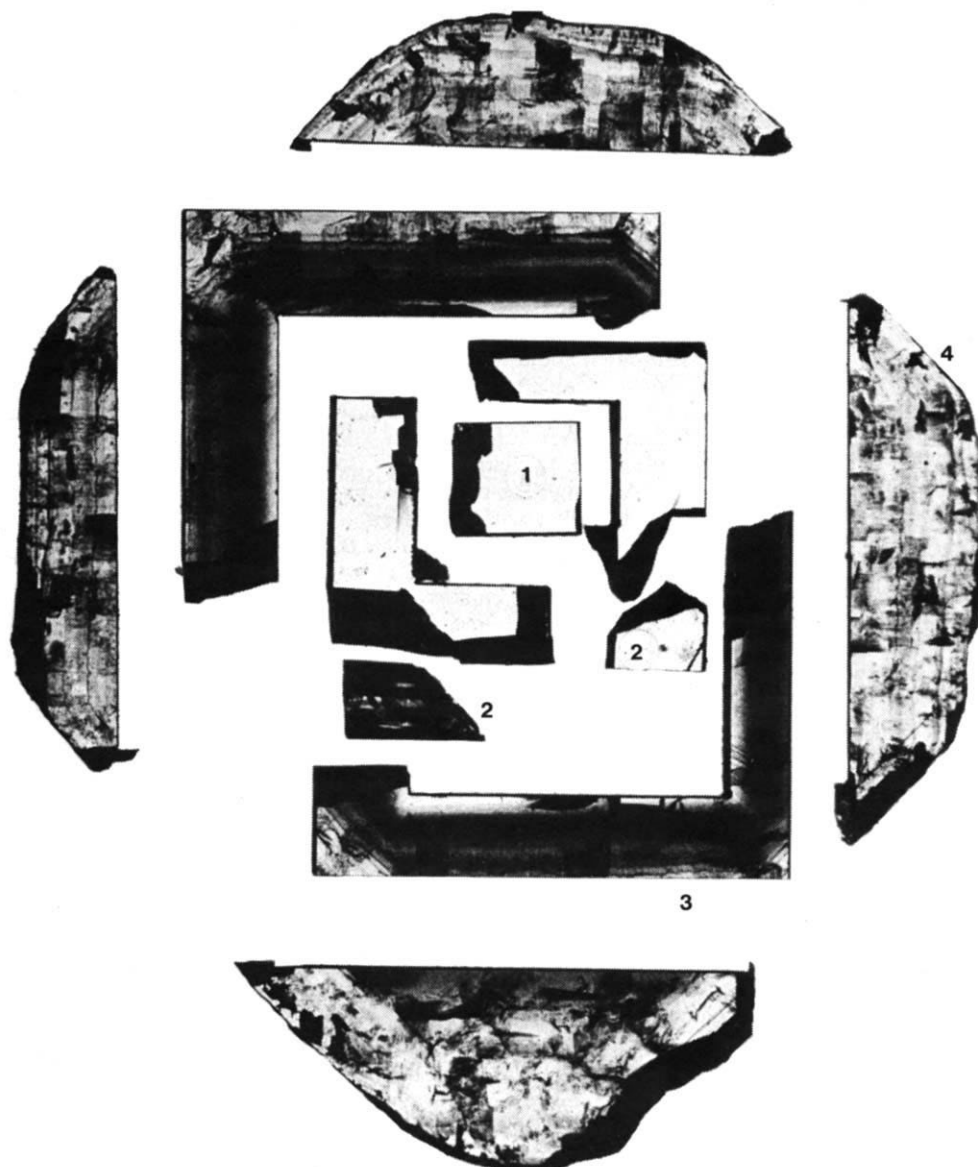


Fig. 1 A laser-dissected section (6-mm diameter) from a type I Congo diamond. Cuts (30 μm wide) were made in concentric rectangles using a Q-switched neodymium-YAG laser delivering 1.3 mJ focused with a 25-mm objective. The {111} growth horizons in the diamond intersect the section as a {110} 'picture frame'. The fragments numbered 1-4 were subjected to stable isotope analysis.

to {111}, all faces being rough compared with octahedral surfaces of transparent diamonds. Observations by Kamiya and Lang⁸ indicate that normal growth was interrupted by deposition of a layer of fine particles. Diamond fibre growth was initiated, and the fibres grew independently with considerable misorientation, and incorporated further particulate impurities in doing so. Coat and core samples have been burned in pure oxygen and the resulting gases analysed by gas chromatography⁹. Nitrogen contents were found to range from 0.047 to 0.148 wt %, with coat-to-core ratios varying from 0.58 to 2.24. In the present work the nitrogen content of 47 specimens of the type I diamonds have been inferred from IR spectra and in particular, the clear core always showed the 7.3 μm platelet line, whereas this feature was always absent from coat material. Laboratory studies indicate that the 7.3- μm platelet line is associated with nitrogen aggregation at higher temperatures indicating that cores have been substantially hotter than the coats. Similarly, all 47 samples studied show the A and B nitrogen lines, but total nitrogen concentration was not constant between coat and core of the individual samples, some showed more in the coat and some more in the core. The sharp 3.22- μm line generally considered to be associated with hydrogen occurred in the spectra of 32 of the 47 samples (eight in the core only, three in the coat only and 21 in both).

It has frequently been debated to what extent the full range (+2.7 to -34.5%) of carbon isotopic compositions observed for diamonds reflects fractionation¹ or compositions inherited from

the source carbon². The smaller variations observed here for single stones might also be discussed in this context. Intuitively, it seems very unlikely that a regular $\delta^{13}\text{C}$ trend from light at the centre to heavy at the edge can be accounted for by a model which relies on the injection of carbon from isotopically-distinct reservoirs. Growth of a coated diamond from a mantle seed of a different carbon isotopic composition or multistage-formation processes from a different reservoir or one which is changing through mixing, might be expected to give a random isotope distribution. On the other hand, processes which rely on equilibrium fractionation during gas-gas or gas-solid interactions, fractionation from a confined volume according to a Rayleigh distillation model, or diffusion, might be expected to produce a regular pattern. Fractionation effects have been discussed extensively by Deines² who considered a large number of models to evaluate the effects of gas composition, initial reservoir composition and the interrelated roles of temperature and pressure. He concluded that fractionation between carbon species was too small to account for the observed total range of carbon isotopic compositions in all diamonds. However, the magnitude of the fractionation calculated could conceivably account for the variations observed here. Although we have no quantifiable evidence which would tend to favour any one of the processes discussed by Deines, the regular changes in isotopic compositions in coated stones are at least consistent with formation from a predominantly carbon dioxide source according to a Rayleigh distillation process.

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Rare-earth elements and uranium in high-temperature solutions from East Pacific Rise hydrothermal vent field (13°N)

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The mobility of rare-earth elements (REE) and U during hydrothermal alteration of the basalts at spreading centres has long been a matter of concern because of its bearing on the evolution and recycling of the oceanic crust^{1–6}. Previous approaches to this problem have been indirect, through studies on altered dredged basalts or ophiolites. We report here sampling of hydrothermal vent waters from the East Pacific Rise (EPR) at 13°N^{7,8}, which provides the first direct evidence of REE-enriched solutions which, however, leave the budget of these elements in the crust and the ocean rather unmodified. In contrast, uranium, like magnesium, is quantitatively taken up from the seawater during the hydrothermal process.

In January 1982, the diving submersible *Cyana* discovered a new field of bottom hydrothermal activity along the EPR at 13°N⁷. At a depth of ~2,600 m, closely-spaced vents spout solutions rich in Cu, Zn and Fe at temperatures in excess of 300°C and pH as low as 3.8 (ref. 8). Graphite bottles were used by the submersible to collect litre-sized samples of solutions from two vent groups located ~6 km apart at 12°50'30"N–103°57'10"W and 12°46'50"N–103°56'05"W, hereafter referred to as the northern site (NS) and southern site (SS). The NS measured temperatures are ~320°C. At SS, the measurement failed but indication of a 284°C temperature comes from a nearby vent, while 320°C was measured some 30 m to the north. Samples of glassy basalts were also collected from the close vicinity of the vents.

Five non-acidified samples were selected (three at NS, two at SS) for REE and U analysis by isotope dilution. Mixed spikes of REE and U were added in the laboratory to 5–15 ml of the sample which was heated to dryness. A combination of cation-exchange and reversed-phase chromatography was used to purify and split the REE in two groups (Ce–Eu, Gd–Yb). Uranium was extracted on anion-exchange columns. The results and the blanks are reported in Table 1. The accuracy of measurement is believed to be ~5% for basalt REE and solution light (L) REE analysis. Due to blank limitations, Yb in water samples is uncertain by ~2 pg per g, and Ce by 10 pg per g. All U analyses are thought to be correct within 3%. These figures refer to the separation procedure only. Adsorption of REE on the container walls cannot be simply dismissed; however, a first indication comes from an early incomplete analysis of sample 24G2 of which a large volume (~400 ml)

has been acidified for 4 days in its own container before LREE measurements. Agreement within 10% of the small-sample procedure results was obtained. In addition, the duplicate analysis of sample 28G0 involved acid leaching of the vial: its Ce value is off by 15% but agreement is good for the other elements.

The basalts present the nearly flat, LREE depleted spectrum common in tholeiites (Fig. 1). In contrast, the hydrothermal solutions are strongly enriched in LREE relative to seawater and display a pronounced positive Eu anomaly. The Mg contents (Table 1) suggest that two NS solutions out of three are 90–95% pure hydrothermal solutions (assuming Mg = 0 in the endmember) while this proportion falls to ~50% at the SS. The lack of a fully regular variation between the REE concentrations and dilution index (Mg) may suggest sampling or analytical problems. The level of contamination within the syringe and during the analytical procedure may be checked using the 24G0 analysis. The Mg content of this sample is indicative of ~95% of seawater and suggests a 'miss' of the sampling process. Its REE contents are less precisely known, but are low enough to ensure that contamination effects are negligible for richer samples. Precipitation of small amounts of anhydrite, which is known to incorporate significant amounts of REE⁹, is a more realistic explanation: the values presented in Table 1 should, accordingly, be taken as the minima.

The present results suggest that, except for fairly high water/rock ratios, the amount of REE driven off from the basalt during hydrothermal alteration is exceedingly small. The rock/water concentration ratios are so high (Table 1) that water concentrations are likely to be buffered by the conduit rocks. The ⁸⁷Sr/⁸⁶Sr ratio of 0.7041 reported for both the NS and the SS Mg-free endmember suggests a water/rock ratio of about 5 (ref. 8). This value is probably not completely relevant for REE but confirms that hydrothermal alteration such as observed at 13°N cannot change the REE pattern of abyssal basalt^{1,10}. In particular, Sm–Nd model ages and $\epsilon_{Nd}(T)$ are not affected in the process and no negative Eu anomaly is generated.

This is in apparent conflict with the conclusion reached by Ludden and Thompson¹¹ or Juteau *et al.*¹², who found a significant change of the LREE during basalt palagonitization. As pointed out by Staudigel and Hart¹⁰, the contradiction is

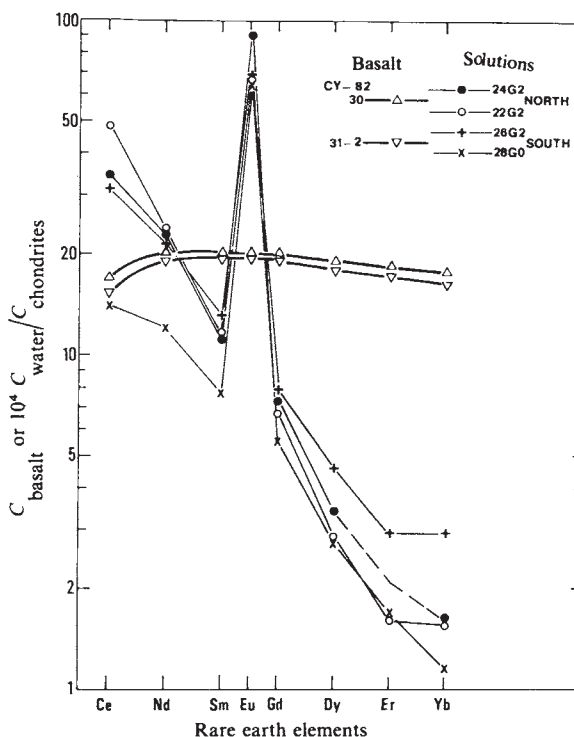


Fig. 1 REE results on basalts and hydrothermal water samples from the EPR 13°N sites. Samples 24G0 has been omitted not to excessively compress the whole variation range.

Table 1 Analytical data on hydrothermal solutions and basalts from the 13° N EPR sites

	Northern site					Southern site				Hydrothermal flux† (10 ⁶ g yr ⁻¹)
	Blank (pg)	24G2 (pg per g)	22G2 (pg per g)	24G0 (pg per g)	Basalt 30 (p.p.m.)	26G2 (pg per g)	28G0 (pg per g)	28G0* (pg per g)	Basalt 31-2 (p.p.m.)	
Ce	215	2,190	3,100	160	10.80	1,980	894	1,001	9.84	7
Nd	70	1,120	1,110	82	9.75	1,015	571	576	9.19	3.5
Sm	75	179	177	19	3.18	205	119	118	3.04	0.6
Eu	12	528	388	18	1.18	395	378	371	1.15	1.7
Gd	(50)	150	136	~5	4.15	162	114	103	3.98	0.5
Dy	45	88	72	3	4.87	117	70	73	4.67	0.3
Er	50	—	27	2.5	3.07	49	28	28	2.95	0.15
Yb	35	27	26	1.8	2.94	49	19	20	2.74	0.1
U	120	158	312	3,185	0.077	1,370	1,050	—	0.082	-10
Eu/Eu*	—	9.85	7.65	~5.6	0.99	6.63	9.92	—	1.01	—
pH	—	3.9	3.8	6.9	—	4.8	4.8	—	—	—
Mg (p.p.m.)	—	83	157	1,154	—	620	600	—	—	—
Mg/U × 10 ⁻³	—	525	500	360	—	450	570	—	—	—

* Duplicated analysis with acid leaching of the container walls.

† Using 7.5×10^{-9} ml g⁻¹ of ³He on 24 G2 (L. Merlivat, unpublished results) and a total ³He flux of 2.4×10^7 ml yr⁻¹ (ref. 5).

only apparent: the ridge axis hydrothermal processes affect a thick section of the oceanic crust with rather low water/rock ratios. In contrast, the palagonitization occurs at the uppermost basalt/water interface in presence of a virtually unlimited amount of water and the relevance of data from this particular environment to the bulk of the oceanic crust is only marginal.

The pronounced fractionation of REE between the basalt and hydrothermal solutions is not likely to be attributable to a simple dissolution of the solid material. As shown by Denis and Michard¹³ for low-temperature conditions, such a process does not fractionate elements entering the same crystallographic site. In contrast, concentration ratios may be heavily affected by the precipitation of new solid phases. At the temperature of the hydrothermal interaction, the REE fractionation should be related to the formation of metamorphic minerals. The temperature–pressure dependence of silica solubility suggests⁸ that, at 13 °N this process occurs at temperatures higher than 350 °C and depth >1 km below the sea floor. These conditions are representative of the greenschist metamorphic facies and fit nicely within the ophiolitic model for a reaction with the effusive rocks from the upper levels. The prevailing mineralogical assemblage is likely to be dominated by quartz, albite, epidote, chlorite, actinolite, and sphene¹⁴. The anomalously high Eu and Sr (ref. 8) release into the fluids suggests that plagioclase is not a critical phase in buffering the REE, while actinolite or sphene appear to be better candidates for this role.

There is finally a strong similarity of the REE spectra in hydrothermal solutions with those from the Red Sea metalliferous sediments^{15–17} including the LREE enrichment and huge positive Eu anomaly. The abundance of iron hydroxides in these samples (goethite, limonite) makes it likely that REE have been scavenged during oxidation and sedimentation of hydrothermal sulphides with little or no fractionation.

Uranium mimics the behaviour of magnesium but the Mg/U ratio changes from ~500 in high-temperature solutions to ~380 in the ambient seawater. It is quantitatively taken up from seawater by the basalt, as previously suggested^{2–4,18,19}, yet the 13° N site provides a first direct evidence. The roughness of the U–Mg correlation is reminiscent of the REE behaviour. As we suggested earlier for these elements, U may have been partially removed by mineral precipitation (anhydrite?) at the subsurface level^{20,21}. With a water/rock ratio of 5, ~15 parts per 10⁹ (p.p.b.) of seawater-derived U are added to the basalt. If the concentration measured in surface basalts are relevant to the upper oceanic crust, this demands a 20% increase of its U content. The actual variation range of U concentrations encompassed by dredged tholeiitic basalts may extend over several hundredths of a per cent^{1,18}. The effect of magmatic processes, either fractional crystallization or variable degrees of partial melting, has been repeatedly dismissed as causing insufficient variations. It is likely that the addition of U to the oceanic crust by ridge crest hydrothermalism represents only the early part

of its enrichment, which from the ridges to subduction zones appears to be a continuous process, at least for the uppermost basaltic layers. Implications that these processes have for constraining the long-term recycling of oceanic crust and sediments in the mantle through lead isotope and ³He/⁴He ratios have been discussed previously^{3,5,22}.

A further grasp on this problem would be obtained if Pb and Th data were available on hydrothermal solution, which could help clarify the long-term evolution of lead isotope ratios in the subducted lithosphere. Lead is likely to be severely leached from the basalts. Whereas the presence of sulphide precipitates in sample containers would make a direct measurement on hydrothermal solutions unreliable, Pb contents up to 2,000 p.p.m. have been reported for 21° N hydrothermal sulphide deposits and basalts from the same site contain only ~500 p.p.b. (refs 23, 24). If the sulphides precipitation is nearly conservative for the Pb/Fe ratio, this means, for the current water/rock ratio and Fe content of the high-temperature component⁸, that a large part of the basaltic lead goes into the hydrothermal solutions. On the other hand, Th is likely to follow a REE-like behaviour and accordingly should not be appreciably leached from the basalt. All these processes concur to a large increase of the U/Pb and presumably Th/Pb ratio in the hydrothermally altered crust.

Conceivably, studies on ophiolitic sequences could also help in constraining the U–Th–Pb budget of the subducted crust. Chen and Pallister²⁵, however, found that, despite high U/Pb values, the three elements are concomitantly enriched in the uppermost layers of the Samail ophiolite. It is clear that Th, Pb and probably some U must have been introduced from the overlying sediment, that is, in a significantly off-ridge position or even during ophiolite emplacement. Hence, the geological site, where the subducted slabs acquire their final U–Th–Pb characteristics, is still uncertain (see ref. 26).

The present data are also of interest for the REE oceanic cycle. If one makes the extreme assumption that the dilution of these elements is conservative (that is neglecting scavenging by particulates) and using both the ³He value obtained by L. Merlivat (personal communication) on sample 24G2 (7.5×10^{-9} ml STP g⁻¹) and the global hydrothermal ³He flux estimated by Jenkins *et al.*²⁷ (2.4×10^7 ml STP g⁻¹), the hydrothermal flux of REE can be calculated (Table 1). A considerable uncertainty resides in the representativity of this particular ³He datum since for an equal number of calories, the 24G2 vent emits some 50 times more ³He than other vents from the Galapagos and EPR at 21° N (refs 27, 28). This anomalous behaviour is confirmed by the unprecedented chemical anomalies such as for chlorine or strontium, observed in the 13° N vent fields⁸. Keeping these restrictions in mind, however, the hydrothermal REE component in the seawater is orders of magnitude less than the continent-derived components. This seems to substantiate the contention of Elderfield and Gieskes²⁹ who attribute the near bottom REE gradients to a diagenetic

remobilization from sediments and similar arguments of Piepgras and Wasserburg³⁰ from Nd isotopic compositions in the South Pacific water column.

The magnitude of REE scavenging by particulates and among them iron and manganese hydroxides possibly associated with biogenic detritus is insufficiently understood at this moment.³ He from the hydrothermal plumes has been detected hundredths of kilometres away from the spreading centres and extends over most of the deep water column⁵. Only a small proportion (<10% according to ref. 7) of the hydrothermal transition elements is deposited to form sulphide chimneys, the rest being blown away into the ocean. The progressive oxidation of the metal sulphides from the plume by seawater and organisms is expected to generate abundant iron and manganese hydroxides presenting a strong REE enrichment. An actual example of such a mechanism may be found in the Red Sea metalliferous sediments¹⁵⁻¹⁷.

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Thermal neutrons could be a cause of biological extinctions 65 Myr ago

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Tabulations¹⁻⁴ of the number of genera present before and after the Cretaceous-Tertiary extinctions have revealed certain patterns¹⁻⁶. Marine organisms were much more susceptible to extinction than were freshwater or terrestrial organisms. Benthic marine organisms were affected as much as those organisms that swam or floated. However, some marine genera were unaffected whilst some terrestrial ones were severely affected. Organisms making calcium-containing skeletons were the most sensitive. Among the hypotheses^{2,5-9} proposed for the extinctions is that they were caused by ionizing radiations^{2,8,9}. The hypothesis proposed here is that the pattern of extinction in the fossil record emerged as a consequence of neutron activation of certain elements in organisms and in their environment.

The model for the mechanism of the extinctions consists of four parts. (1) Neutron-producing radiation greatly enhanced over the present background level briefly bombarded the Earth. (2) Thermal neutrons interacted with elements in seawater and in organisms to form radioactive substances. (3) All organisms received an increased dose from ionizing radiations. But those that accumulated Ca or S from seawater, Ca, P, or S from plants selectively received a lethal dose from the decay of ⁴⁵Ca, ³²P and ³⁵S. (4) The evolution that took place in the early Tertiary was facilitated by the mutagenic action of ionizing radiation⁷⁻⁹. The plausibility of this model depends on three factors. First, an examination of the literature showed that a large flux of thermal neutrons on Earth could have resulted from a supernova, one of the perceived sources of cosmic rays. Second, thermal neutrons were shown to produce isotopes in the environment which could lead to the elimination of particular taxonomic groups of organisms. Third, it has been shown that one of these selectively-formed isotopes could cause radiation damage to a type of organism that was susceptible to extinction.

Neutrons are generated by primary cosmic rays in the atmosphere⁹⁻¹⁴. Cosmic radiation at large intensities is thought to arise occasionally from nearby supernovae¹⁵⁻¹⁸ and either from the Sun^{19,20} or from an explosion at the galactic centre^{16,19}. Studies of cosmic-ray tracks in meteorites²¹⁻²³ and in Moon rocks suggest little variability in cosmic-ray flux over the past 50 Myr, but uncertainty over the exposure history of lunar rocks makes it difficult to analyse for variability in cosmic ray bombardment beyond 50 Myr (ref. 21). It thus remains a plausible assumption that a large increase in cosmic ray flux could have occurred about 65 Myr ago.

The energy, E_{cr} , imparted to cosmic rays by a supernova is estimated^{16,24,25} to be in the range of 10^{48} - 10^{56} ergs with an average value of 10^{51} ergs. Explosions at the centre of the Galaxy^{16,19} may produce cosmic rays with a value of 10^{56} ergs. Supernovae occur in our Galaxy about every 50 yr (refs 16, 25) with the more energetic ones, $E_{cr} = 10^{56}$ ergs, occurring every 10^7 - 10^8 yr. The energy, $E_{cr,R}$, of cosmic rays at R cm from a supernova or an explosion at the galactic centre is the subject of erudite propagation models¹⁶ but is approximated for heuristic purposes⁸ by

$$E_{cr,R} = E_{cr}/4\pi R^2 \quad (1)$$

The influence of galactic and solar magnetic fields are ignored. The mean energy, E_{avg} , of a primary cosmic ray at the top of the Earth's atmosphere is 7 GeV (ref. 14). The mean number of nucleons in the primary cosmic ray flux is $0.181 \text{ cm}^{-2} \text{ sr}^{-1} \text{ s}^{-1}$ (ref. 16). In the case of a sudden enhanced cosmic ray flux, a much greater number of the primary cosmic rays would survive collisions with the Earth's atmosphere and would produce nuclear reactions and thermal neutrons on the Earth and its oceans. It seems conservative to assume that the number of thermal neutrons, in the Earth and its oceans, per primary cosmic ray at the top of the Earth's atmosphere would be the same under an augmented cosmic ray flux as it is today.

The flux of thermal neutrons, F_n , at the Earth's surface was estimated from the following equation,

$$F_n = E_{cr,R}m/(tE_{avg}) \quad (2)$$

where $E_{cr,R}$ is from equation (1), m is the number of thermal neutrons produced at the Earth's surface per primary cosmic ray at the top of the atmosphere and t is the time interval over which the cosmic rays arrive. An interval of 365 days was used for the calculations in Fig. 1. Neutrons with thermal energies up to 10 MeV have been measured²⁶ at a flux of 7.3×10^{-3} neutrons $\text{cm}^{-2} \text{ s}^{-1}$. This resulted in an estimate of m at 4×10^{-2} . Using these values, Fig. 1 shows that a large thermal neutron flux at the Earth's surface was a plausible event from a large supernova in the vicinity of the Earth. Also shown in Fig. 1 is the effect of the distance between the supernova or galactic centre explosion on the expected cosmic-ray produced neutron flux.

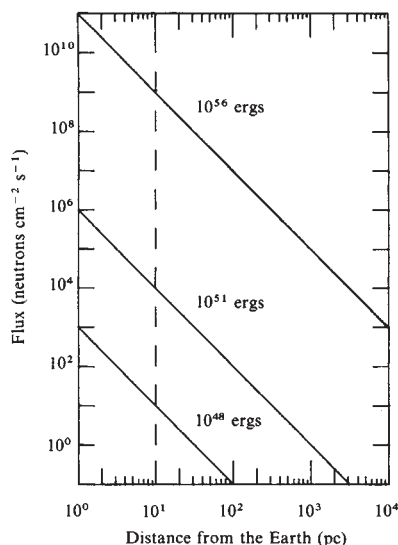


Fig. 1 Estimates, based on a simple propagation model⁸, of the neutron flux at the Earth's surface due to cosmic ray bursts originating at up to 10^4 pc from the Earth. Three different energies of the bursts are shown: 10^{56} ergs is an estimate of the maximum energy imparted to cosmic rays by a supernova or an explosion at the galactic centre^{16,19}; 10^{51} ergs is an estimate¹⁶ for the energy imparted to cosmic rays by the average supernova; and, 10^{48} ergs is an estimate¹⁶ for the lower limit of energy imparted to cosmic rays or the maximum energy²⁴ imparted by certain types of supernovae. Each curve was generated from equation (2) with $m = 0.04$ and $t = 365$ days. At distances within 10 pc (to the left of the dashed line) of the Solar System, supernovae are estimated²⁵ to occur with a 0.01–0.5 probability every 10^8 yr. A neutron flux of 5.5×10^9 neutrons $\text{cm}^{-2} \text{s}^{-1}$ for 1 yr could produce ^{45}Ca at 26,230 d.p.m. g^{-1} of seawater (ignoring, for heuristic purposes, ocean mixing). As shown in Fig. 3, this amount of ^{45}Ca would give radiation damage to certain types of organism.

Neutron bombardment of seawater produces radioactive elements²⁷ that would be incorporated into organisms; and, neutron activation of freshwater like that in the Columbia River produces small amounts of such elements²⁷. The types and amounts of nuclides formed in seawater, freshwater and plants for a range of neutron fluxes were calculated using the neutron activation equation^{27,28}. Literature values were used for the elemental composition of seawater²⁹, of freshwater³⁰, and of plants³¹. The flux of thermal neutrons (1×10^{10} neutrons $\text{cm}^{-2} \text{s}^{-1}$) and irradiation time (1 day) were chosen for the results in Fig. 2 to demonstrate the selective production of bioavailable radioisotopes. After a few days following a brief irradiation, the principal radioisotopes would be ^{45}Ca , ^{35}S , and ^{32}P .

Studies of radionuclides released to the environment from nuclear weapons tests and from the nuclear fuel cycle provide examples of how organisms concentrate radionuclides and of how these move through food chains^{32–34}. For example, man-made radionuclides entering the shallow waters of the seas are rapidly taken to benthos by faecal pellets³⁵ and by discarded mounds³⁶. Thus, radioactivity induced in shallow parts of seas by a world-wide neutron activation would move rapidly to benthos. In fresh waters, organisms were shielded by the water and significant concentrations of activation products were not formed. This could explain how in the Cretaceous–Tertiary boundary interval benthic marine organisms were affected but benthic freshwater organisms were spared.

Other patterns in the extinctions followed from the model. From tabulations given elsewhere^{1–4}, it is apparent^{5,6} that siliceous organisms (radiolarians and diatoms) were unaffected by the extinction event. Whereas those organisms containing calcium carbonate were decimated. Coccolithophores were reduced from 43 genera to 4 and foraminifera from 18 to 3 by the extinction event^{1–4}. The production of ^{45}Ca in seawater could account for the demise of these organisms. They would

have accumulated radioactive calcium from seawater and thus received a large dose from the β particles of ^{45}Ca . Although not very abundant (2%), ^{44}Ca activates to ^{45}Ca which decays with a half life of 165 days. Organisms containing only silicates were spared because the concentration of silicon in seawater is 3 mg l^{-1} compared with that of calcium at 410 mg l^{-1} , because silicon interacts with neutrons less than does calcium, and because ^{31}Si decays long before much of it can be incorporated by siliceous species. The relationship of extinction to mineralogy extends to benthos with an apparent sensitivity to extinction among benthic calcite-forming organisms^{1–6}. Such data need to be examined in more detail to determine how pressure-dependent changes in the mineralogy of marine organisms might have influenced the incorporation of ^{45}Ca . And, how such pressure changes affecting the dissolution of carbonates might affect the amount of ^{45}Ca transported to benthos by sedimentation. These and other factors would contribute to an attenuation of ^{45}Ca with depth.

The extinction of land animals with large bones, containing not only Ca but also P, followed from the hypothesized neutron activation. ^{32}P and ^{45}Ca would have formed directly in the bones of organisms and, perhaps more significantly, in their foodstuffs such as plants (Fig. 2). It seems plausible that organisms with large bones and hence an active mineral metabolism received a large dose from the decay of such induced and incorporated radioactivity. It may also be relevant that many dinosaurs laid Ca-containing eggs³⁷.

The magnitude of neutron flux used in the calculations for Fig. 2 was chosen to illustrate the selective production of biologically important isotopes. As discussed above, a supernova could produce cosmic rays and hence neutrons at fluxes

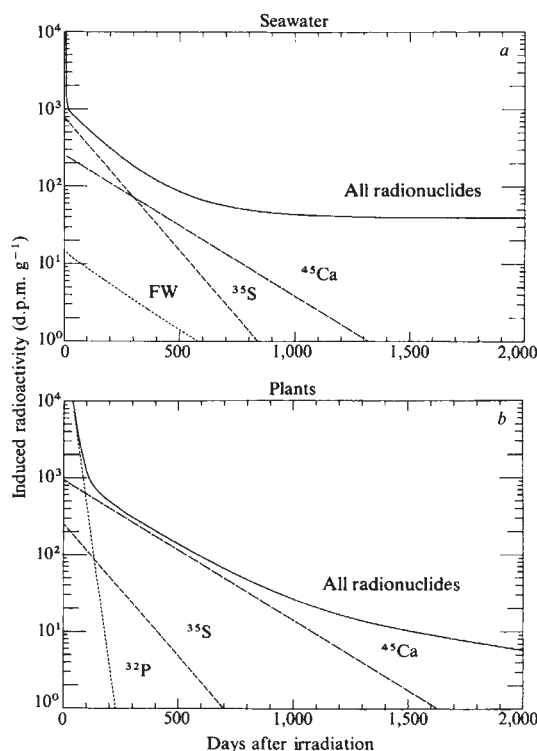


Fig. 2 The amount of induced radioactivity in d.p.m. in 1 g of seawater, in 1 g wet weight of plant tissue, and in 1 g of freshwater was calculated using a brief hypothetical exposure of 1×10^{10} neutrons $\text{cm}^{-2} \text{s}^{-1}$ for 1 day. *a*, In fresh water (FW) there would be comparatively little radioactivity formed. In contrast, the total induced radioactivity in seawater would be quite large. Much of the activity would decay rapidly. For several years following irradiation, the remaining activity would be accounted for by ^{45}Ca and ^{35}S . *b*, Irradiation of plant tissues would result in the persistence of ^{45}Ca and ^{35}S in a manner similar to that seen in seawater. In addition, ^{32}P formation, as shown, would be significant in plant tissues.

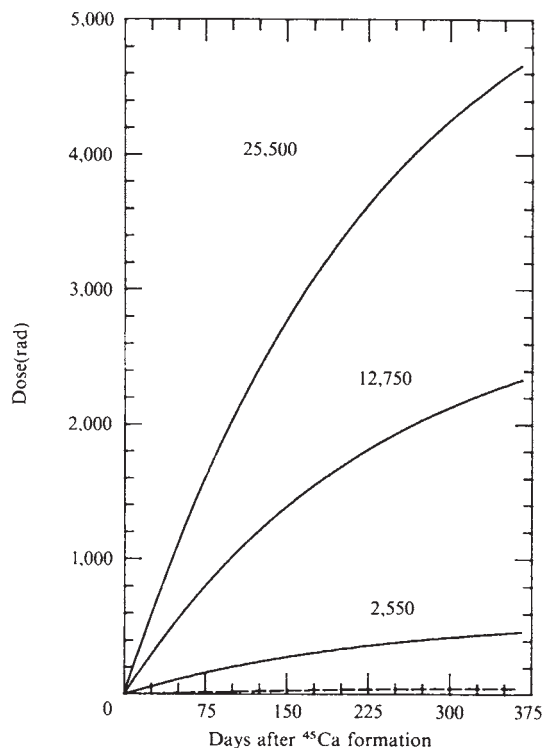


Fig. 3 The approximate dose that would be received by a coccolithophore such as *Coccolithus huxleyi* following the uptake of ^{45}Ca from seawater. The lowest dashed line is for the uptake of ^{45}Ca at 255 d.p.m. g^{-1} of seawater. The other curves are for 10, 50 and 100 times this amount of ^{45}Ca .

even several orders of magnitude greater than that used in Fig. 2. The following calculation illustrates that within the range of possible neutron fluxes, levels of ^{45}Ca could form to give a large dose of radiation to a coccolithophore—one of the genera that was decimated 65 Myr ago. ^{45}Ca decays with a half life of 165 days and emits a β particle having an average energy, E_β , of 0.076 MeV. The dose, D , in erg g^{-1} of substance is slightly overestimated by³⁸

$$D = AE_\beta(1 - \exp(-\lambda t))/\lambda \quad (3)$$

where A is the disintegration $\text{s}^{-1} \text{g}^{-1}$ of substance, λ the decay constant, and t is the time over which the dose is deposited. One kind of coccolithophore³⁹, *Coccolithus huxleyi*, is 5 μm in diameter and contains 15 μg of Ca. If these organisms were to achieve the same specific activity of ^{45}Ca as would be produced in seawater by neutron activation, and if the β -particles were to lose all of their energy to the organisms, then they would receive a dose as was calculated with equation (3) and is shown in Fig. 3. The calculation showed that a flux of 10^{12} neutrons $\text{cm}^{-2} \text{s}^{-1}$ would produce 25,500 d.p.m. of ^{45}Ca in seawater and would give to such a coccolithophore a dose of 4,700 rad in 1 yr. This dose is in the range of 4,000–6,000 rad that reduces populations of marine bacteria to 37% survivors⁴⁰.

Radionuclides formed by the proposed neutron activation would have decayed to undetectable levels in 65 Myr. Changes in the amounts of stable elements formed or depleted by neutron interactions with Earth elements, may be detectable. Data on the stable element composition of the Cretaceous–Tertiary boundary sediments are used to support the idea that material entered the Earth as an asteroid⁴¹. The mechanisms that lead to the trace-element composition of sediments include the interaction of minerals from dead organisms with the ocean and sediment environment⁴². There are differences between the role of carbonate and silicate minerals⁴². Thus the hypothesized neutron activation of seawater that led to the selective demise of calcareous organisms, could have also affected indirectly the trace element composition of sediments. First, there could have been an alteration in the ratio of the amount

of silicate to the amount of carbonate sedimenting. Second, as a consequence of the first event, there would have been an alteration in the suite of trace elements being extracted from seawater. Finally, there may have been a change in the trace element composition of seawater itself as a consequence of the neutron reactions. Many of the trace elements at enhanced levels in the sediments of the Cretaceous–Tertiary transition⁴¹ can be formed as the decay products of neutron-activated seawater. A proper testing of the hypothesis that the enhanced elements were the result of neutron activation, would require additional knowledge of the biogeochemical cycles of these elements.

The argument⁴¹ in support of the asteroid impact hypothesis centres around the unusual abundance of iridium in the Cretaceous–Tertiary boundary layer sediments. That the iridium may have arisen from a supernova was rejected⁴¹ in view of the presently proposed mechanisms of supernovae and their estimated probability of occurrence. The asteroid impact hypothesis appears further bolstered by the finding of an iridium anomaly in non-marine sediments of about the same age⁴³. Not much, however, is known about the biogeochemistry, weathering, solubilities and transport of platinum group metals⁴⁴. There are few measurements of the concentrations of iridium and osmium in seawater. Osmium and iridium in deep-sea sediments are found in variable ratios, their abundance is correlated with the sedimentation rate, and possibly on the carbonate content of the sediments⁴⁵. Thus the model of neutron activation as the cause of extinctions is not negated by the data of trace-element composition in the Cretaceous–Tertiary boundary sediments.

In future work it may be possible to calculate the magnitude and duration of neutron fluxes that could have produced extinctions from the radiosensitivity of organisms.

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Selective impairment of educationally subnormal children's delayed memory for text

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We have tested the ability of educationally subnormal (ESN) children to recall information from a short story, both immediately, and after a delay of 1 week. Their performance was compared with that of two normal groups: one matched for chronological age, the other matched for reading age. In the immediate test, despite differences in the overall level of recall, all three groups showed a similar pattern of recall for important and unimportant information. However, while both normal groups showed a reliable bias towards recalling more important than unimportant information in the delayed test, the ESN group showed no such effect of importance in their delayed recall. This finding suggests that ESN children may fail to utilize differences in importance in consolidating their memory for text.

Efficient memory for a piece of text is not a passive process: it demands the construction of an integrated representation of the ideas and events in that text. The central, or more important, ideas of the text must be identified in order to build such a representation. Information rated as important is better retained by even quite young children¹. Further, the amount of information forgotten from a story after a delay is a function of the importance of that information: more unimportant than important information is forgotten after a delay of 1 week, although the magnitude of this effect depends on the time of day of the original presentation². A study of text memory in ESN children³ has shown that they distinguish between important and unimportant information in their immediate recall of text, despite the fact that they are generally held to suffer from an inability to focus attention on the most relevant features in learning tasks^{4,5}. However, the question of whether subnormal children make use of such information in consolidating their text memory has not been addressed. If ESN children are less able to select important information as the basis for consolidating an integrated memory representation of a text, this would be expected to be associated with relatively poor recall of important information after a delay.

Three groups of children participated in the experiment. The ESN group consisted of 20 children (mean age = 11.5 yr, s.d. = 0.3 yr; mean reading age = 8.1 yr, s.d. = 1.3 yr). Any children with obvious brain damage or physiological abnormalities were excluded from the sample, as were maladjusted and hyperactive children, and those whose first language was not English. The group was thus (as far as it was possible to ascertain) comprised of 'cultural-familial retardates'. There were two control groups: one group of 20 children matched with the ESN group for chronological age (NCA), and having reading ages commensurate with their chronological age (mean age and reading age = 11.5 yr, s.d. = 0.3 yr), and a second group matched with the ESN group for reading ability (NRA), and having reading ages commensurate with their chronological age (mean age and

reading age = 8.1 yr, s.d. = 1.3 yr). Reading ages were based on recent assessments by the schools, using the Schonell graded word test⁶.

A short story (Reynard the Fox by Edward Korel), that was unfamiliar to the children, was tape-recorded for presentation. The story is an adaptation of a fable, and was chosen for its appeal across the age and ability range. A number of questions about the story were generated, and two matched groups of 18 were finally selected for inclusion in the questionnaires. The questions were varied according to whether or not they addressed important aspects of the story, and whether they required an inference. Each question was accompanied by four alternative answers. Any questions that were answered completely correctly or completely incorrectly, in a pilot study using ESN children, were omitted. Six undergraduate students were given a transcription of the story, together with a correctly completed questionnaire. They were asked to rate the importance of the information tapped by each question to an understanding of the story, using 10-cm visual analogue scales. They also rated the questions according to the level of inferential processing they required. There was a highly significant degree of concordance in the ratings for each question in terms of importance (Kendall's $W = 0.44$, $P < 0.001$), and inference ($W = 0.47$, $P < 0.001$). The mean ratings given to each question were, therefore, taken as indices of its importance and inference level.

Two parallel questionnaires were compiled with the questions matched for importance and inference ratings. In each questionnaire, the questions were presented in the same order as the events to which they referred occurred in the story. The subjects were tested in groups of five, in a quiet room. They listened to the tape recording of the story, which lasted ~12 min, and were presented with one questionnaire for completion immediately after the presentation; 1 week later they completed the second questionnaire. At each recall test, the method of recording answers was explained to the children, and the questions were read aloud to them with a pause between questions while all children recorded their response. Both test sessions took place between 9.30 and 10.30 am.

A preliminary analysis of the data, in which level of inference was included as a factor, revealed no differential effects of the inference manipulation between the normal and ESN groups. Consequently, this factor was ignored in all further analyses. An analysis of variance, in which the nine least important questions were compared with the nine most important ones, was performed on the data. The percentages of correct answers to the questions are shown in Fig. 1. There was a significant difference between the three groups in their overall ability to answer the questions ($P < 0.001$); fewer questions were answered after a delay ($P < 0.001$), and overall fewer low-important than high-important questions were answered correctly ($P < 0.001$). The ESN group's recall of high and low-important information was almost equal after a delay, whereas the control groups recalled relatively more high-important information with a delay. This differential effect between level of importance, delay and groups was highly reliable ($P < 0.001$).

To explore further this differential effect, separate analyses of variance were performed comparing each pair of groups. The analyses revealed that both normal groups showed a similar pattern of selective retention to one another, but a significantly different pattern to that of the ESN group (ESN versus NCA, $P < 0.001$; ESN versus NRA $P < 0.001$).

There were no significant correlations between the rated importance of a question and the proportion of subjects who correctly recalled it for any of the groups on the immediate test. (Our failure to replicate the significant effect of importance on ESN children's immediate retention, reported elsewhere³, probably reflects procedural differences.) However, there were correlations between rated importance and delayed recall for the reading age controls ($r = 0.508$, $P < 0.05$) and for the chronological age controls ($r = 0.615$, $P < 0.01$), but not for the ESN group ($r = 0.046$). This pattern of correlations further

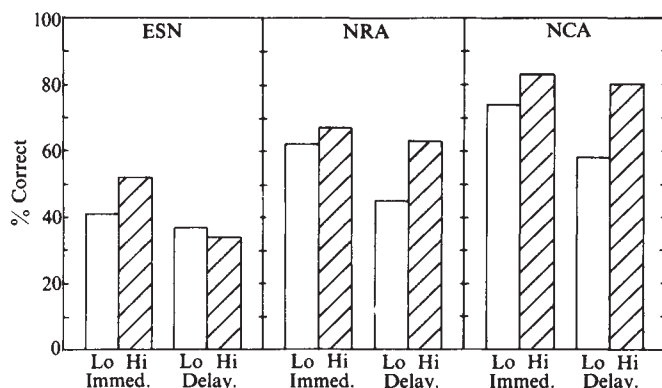


Fig. 1 The percentage of questions answered correctly by the ESN and normal groups, as a function of the rated level of importance (high or low) and the recall condition (immediate or delayed). NRA, Normals matched for reading ability; NCA, normals matched for chronological age.

supports the idea that normal children better recall more important information after a delay, whereas ESN children show no such pattern of selective retention.

These results provide support for the importance by delay interaction reported in normal children², and demonstrate that normal children of different ages are similarly sensitive to the importance level of information, despite overall differences in recall level. In contrast, the ESN group showed not only poorer overall levels of recall than either of the control groups, but also a qualitatively different pattern of forgetting compared to normals. This suggests that their deficit is due to more than a simple quantitative retardation of memory processes. All groups showed similar patterns of recall for important and unimportant information in the immediate test, so that the differential patterns of delayed recall cannot be accounted for by differences in the ESN group's immediate memory. Unlike the normal groups, however, the ESN group appear to have failed to use information about levels of importance during the process of consolidating their memory for the text. After 1 week their memory for important information was no better than that for more trivial aspects of the story. Even the younger control group consolidated the more important information differentially, and showed better recall for such information after a delay. These findings suggest that ESN children fail to focus on the informative aspects of a text that provide the basis for a structured representation in memory. They appear, rather, to consolidate their memory for text without regard to considerations of importance. Indeed, the normal pattern of results was reversed, with ESN children forgetting more high- than low-important information.

One possible explanation of why important items were differentially forgotten by the ESN group is that they relied almost entirely on maintenance processing, even for delayed retention, instead of attempting to build an integrated semantic representation, and that less important items (that is, trivial details) are more amenable to long-term retention in this manner. Whether this failure in the consolidation processes of ESN children is a general feature of their learning disability remains to be determined but remedial procedures, in the area of text understanding at least, might usefully concentrate on training ESN children to construct integrated representations that take account of importance.

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Behavioural dynamics

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Recent proposals^{1,2} suggest that demography can account in full for the observed patterns of species abundance that are characterized by the exponent, β , of the power function³ relating population mean density, μ , to variance, V , where β ranges from <1 to ~ 4 in different species³⁻⁷. We show here that stochastic demographic simulations¹ fail to mimic real data in certain respects because active behaviour is missing from the models and that the behavioural Δ -model^{8,9} corresponds better with data.

Anderson *et al.*¹ use markovian population models to restate the proposition^{10,11} that environmental and demographic stochasticity determine the relationship between the mean and variance. In such models spatial behaviour, including migration, may affect demographic rates, but its effect is indiscriminate because "the emigration of an individual is entirely equivalent to death"¹⁰. In real life the behavioural vector is not random¹², but unique to each species, hence our approach is to model population growth and movement in coordinate space, controlling distance and direction of movement.

Population mean density, μ , is related to variance, V , by a power function³ (Taylor's power law¹³)

$$V = \alpha \mu^{\beta}; \quad (1a)$$

logarithms are used for sample values, m and s^2 (ref. 5), notation after Perry¹⁴,

$$\log s^2 = a + b \log m. \quad (1b)$$

The parameter, b , an 'index of aggregation'³, is the rate of change of log variance with log mean, fitted as a dependent or functional regression, as appropriate^{5,7,14}.

The models of Anderson *et al.* derive from Bartlett's 'population with migration' model¹⁰ in which spatial mean and variance are related by a quadratic¹⁵ ($s^2 = cm + dm^2$). They use models simulating populations growing in 'patches' with no bounds except carrying capacity, K ; conceptual 'size' is used to create mean and variance. Individuals have no spatial coordinates. Migration has no diffusion rate¹⁶, no density-distance function¹⁷⁻¹⁹, no directional component nor any expression for its aggregative component¹². Simulations concentrate on the population growth stage, not the subsequent 'stable' fluctuations seen in the field data.

Anderson's models 1 and 2: Density-dependent parameters lead to quasi-negative binomial distributions ($c = 1$; $d = 1/k$), with b approaching 2 when m diminishes with respect to m^2/k (their Fig. 1a, b), unlike real data^{3,7}. Projected linear regressions would have different intercepts, $\log a$, and it is these that are determined by the demographic parameters, not b . Models 1 and 2 lead to negative binomial distributions in which k is constant. We²⁰ and others²¹⁻²³, however, have clearly demonstrated that k is not constant or linear in real data.

Anderson's model 3: Density-dependent feedback destroys the $b = 2$ condition in models 1 and 2 by eroding aggregation as density increases. These processes set limits for the linear

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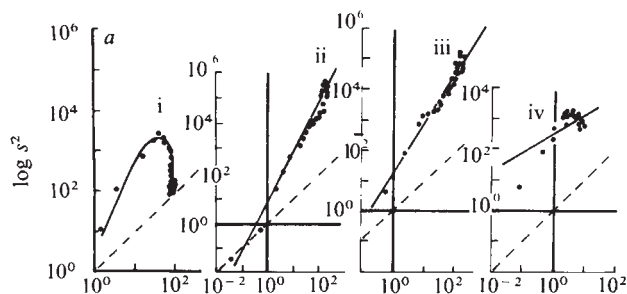
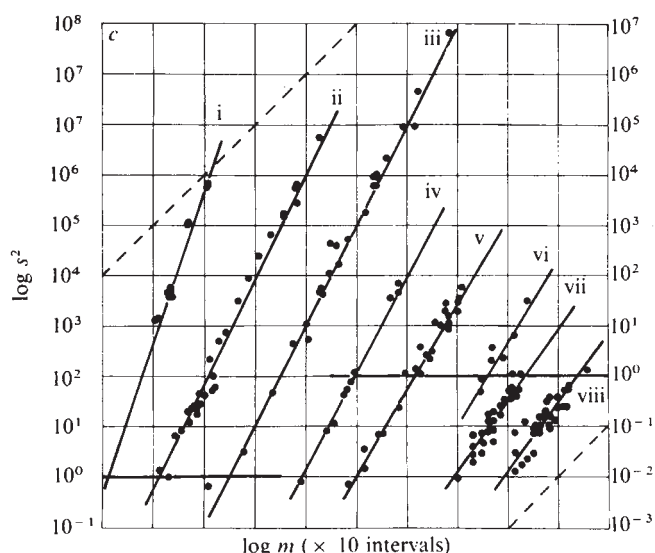
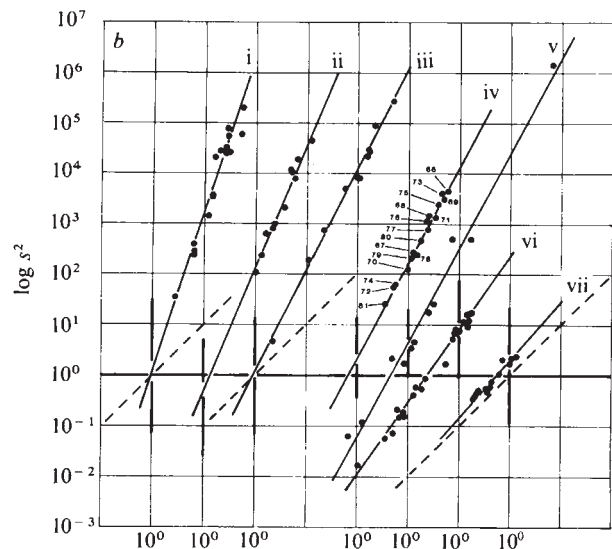


Fig. 1 *a*, $\log s^2 \times \log m$ plots of simulations from the Anderson models¹. (i) model 3; density-dependent mortality in a homogeneous environment (nonlinear regression). This does not occur in natural populations. A possible example quoted by Anderson *et al.*¹, *Microtus pennsylvanicus*, is inappropriate because the relationship is an artefact of the presence/absence nature of the trap used³⁷. (ii), (iii) Model 4; density-dependent mortality in a heterogeneous environment. Regressions are almost linear but still sigmoid, even after means have been taken; with slopes of $b = 1.91$ (ii) and 1.69 (iii). (iv) Model 4; density-dependent mortality in a relatively homogeneous environment (non-linear regression). The smaller the variance of the carrying capacity, the more homogeneous the simulated environment and the greater the similarity with model 3 (Fig. 1ai). *b*, The range of spatial regression coefficients, b_s , fitted as functional linear regressions in logs, to field data⁷ correspond well with the Δ -model and with similar linearity. Consecutive annual data points in field data are not sequentially related (see (iv)) as they are in the Anderson models (*a*) but not in the Δ -model (*c*). During the period of sampling (1962–81)⁶ growth rates, phenology, natural enemies and environments differed widely. (i) *Lycophotia varia*: $b_s = 3.02$, a moth; (ii) *Thelaxes dryophila*: $b_s = 2.42$, an aphid; (iii) *Cavariella pastinaceae*: $b_s = 1.99$, an aphid; (iv) *Plusia gamma*: $b_s = 1.89$, a moth, samples dated from 1966–81; (v) *Aploneura lentisci*: $b_s = 1.87$, an aphid; (vi) woodcock: $b_s = 1.45$, a bird; (vii) *Ophrys apifera*: $b_s = 1.15$, an orchid⁵. The low value of this and other plant species suggests that the more sophisticated animal behaviour is mainly responsible for the higher values of b_s , rather than demographic parameters alone. *c*, Simulations from the behavioural Δ -model^{8,9,18} are always linear. Replicate simulations (ii) and (iii); (iv) and (v); (vii) and (viii) have similar b values even when reproductive parameters differ as in (iv) and (v)³¹. $b =$ (i), 2.93; (ii), 2.19; (iii) 2.06; (iv), 1.87; (v), 1.70; (vi), 1.66; (vii), 1.45; (viii), 1.40.



regression slope of $1 < b < 2$ in conventional theory²⁴, although $b \gg 2$ has long been known in field data^{3,4} and is now well authenticated^{6,7,25}. The output from this model fails to cross the Poisson line; regular, 'under-dispersed' distributions do occur in nature⁵, though commonly only at low densities⁷. The curve in Fig. 1ai has no known equivalent in natural populations. **Anderson's model 4:** Introduces control of environmental heterogeneity, constant throughout a simulation. It is claimed to generate linear regressions between log variance and log mean. Simulations shown have $b = 1.69$ (Fig. 1aii) and 1.91 (Fig. 1aiii); Fig. 1b and c show field data and Δ -model simulations for comparison. Environmental stochasticity attenuates curvature (Fig. 1ai) depending on the variance of carrying capacity. The model generates noticeably autocorrelated sequences of variance/mean pairs. This renders regression invalid and leads to the erroneous claims of slopes greater than 2 for restricted ranges of density near to the 'carrying-capacities' of the 'patches'. The model has not produced sample points with the variance less than the mean, nor evidence that $b > 2$ is possible over a full linear regression as in Fig. 1b and c.

An important defect of the model is that it generates b values that 'vary greatly' between simulation replicates with fixed parameters 'even when sample size is large'¹. Observed values of b do not vary, between replicates, with density range, nor with sample size except over great scale changes such as leaf size to landscapes. Such inconsistencies between simulations

suggest flaws in the initial assumptions or the techniques. In contrast, Δ -model simulations using similar parameters give similar b values (Fig. 1c).

The major elements of the simulations of Anderson *et al.* are: (1) b is highly variable between replicate simulations (that is, population cycles), therefore, real replicate population growth cycles should yield different b values. (2) b is determined by demographic parameters alone, therefore, (a) b s for animals and plants should not differ; (b) change in behaviour should not change b ; (c) b should be maximized in high birth-rate, unstable, r -strategist species, and minimized in K -strategist species. (3) b is claimed to be minimized in homogeneous, and maximized in heterogeneous, environments, and to disappear in a uniform environment. (4) $1 < b < 2$ in conventional demographic theory; Anderson *et al.*'s simulations conform with this.

We now examine these propositions in relation to real data. (1) *Aphis fabae* sampled on different occasions^{26,27} (Fig. 2a) has constant b ; $\log a$ varies with the demographic parameters and sampling method (compare with models 1 and 2). (2) (a) Plant species have notably low b values (0.88–1.48) compared with animals (Fig. 1bvii)⁵. (b) When their behaviour changes in the late fifth instar, mobile larvae of some Trichoptera species actively congregate to pupate under large stones; no 'demographic' change has occurred during this spatial redistribution. Elliott^{23,28} has observed this behaviour which produces a striking and significant increase in b in two species

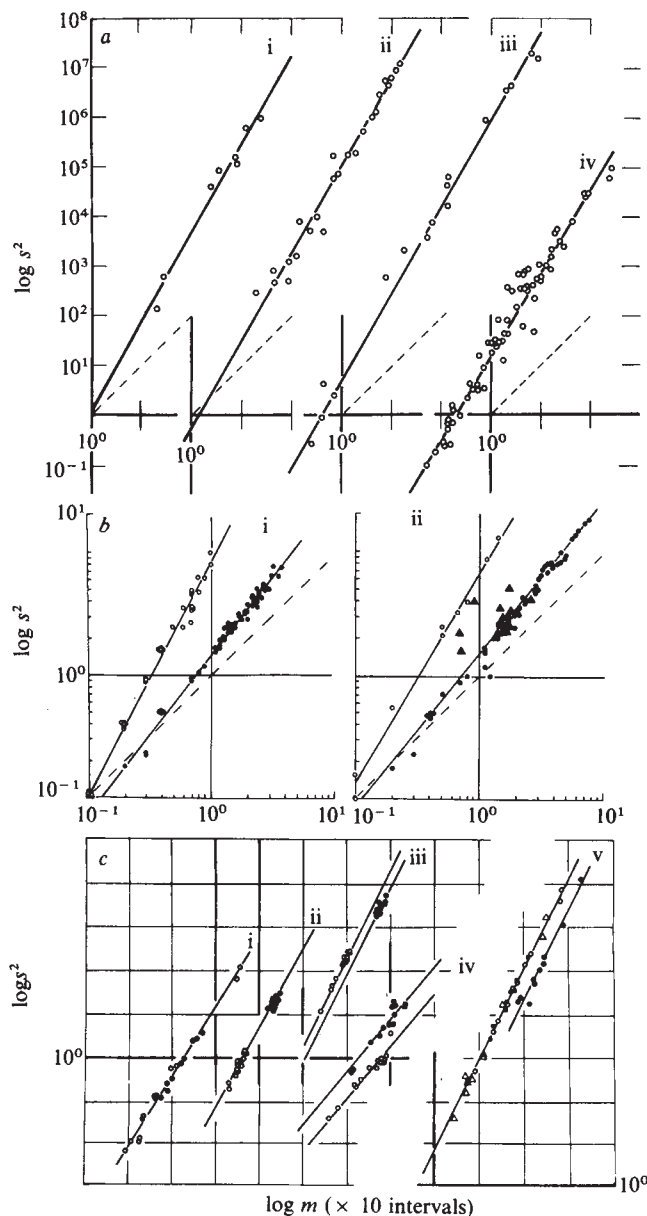


Fig. 2 $\log s^2 \times \log m$ plots of: **a**, The aphid *Aphis fabae* Scop. sampled at intervals during four population cycles growing at different rates, that is, with different demographic parameters, by five different authors using different sampling methods, gives similar b values with different values of $\log a$, (i) Wilding²⁶, (ii) Johnson²⁷, (iii) Banks²⁷ and (iv) Taylor²⁷. Behrendt³⁸ records the same b values in seven populations of Eberswalde (not shown). **b**, Larvae (●) and pupae (○) of three species of caddis flies (i) *Odontocerum albicorne*²⁸ and (ii) *Silo pallipes*²³ and *Philotamus montanum*³⁹ (not shown). Fifth instar larvae (▲) show the progressive change in b , resulting from an observable change in larval migratory behaviour, leading to increased aggregation in their distribution as sedentary pupae, with disproportionately high increase in variance at high local density. **c**, The effect of environment on spatial distribution is shown by a shift in the regression line, changing the intercept, $\log a$, whilst the slope, b , remains constant. Birds were sampled in woodland (●) and farmland (○) throughout Great Britain⁶; (i) stonechat, (ii) tawny owl, (iii) willow warbler and (iv) kestrel. They show progressive, differential responses to environments that are experienced oppositely by different species sampled simultaneously (iii) and (iv). Of 111 bird species, only 8 have different b , in woodland and farmland⁶. (v) The hop aphid; ●, summer virginoparae migrating from *Prunus* spp.; ○, total autumn sexual morphs migrating from hops (△ autumn ♀♀, ▣ autumn ♂♂) reflect the geographical distribution of the two host plants, which is very different, by a change in $\log a$, whilst b , remains constant despite very different demographic parameters¹⁹. The aphid *Myzus persicae* behaves similarly⁴⁰.

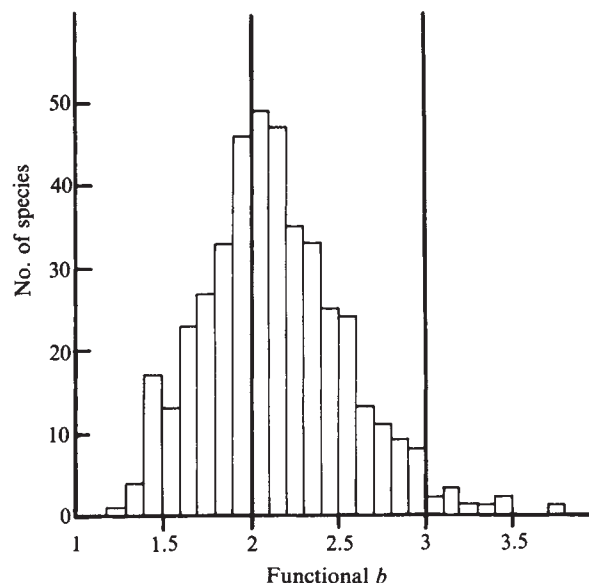


Fig. 3 The frequency distribution of functional b s from 444 different species (birds, moths and aphids) sampled over Great Britain. The mean powers are 2.24 for moths, 2.14 for aphids and 1.79 for birds with an overall mean of 2.12 ± 0.4 (s.d.)⁹.

(Fig. 2b). Behavioural redistribution by age and sex is familiar and these data provide the most unequivocal evidence available that b is affected directly by individual behaviour. (c) Aphididae are extreme r -strategist species, Noctuidae include many r -strategist moths whilst the Geometridae are nearer the K end of the spectrum. Birds may cover a wide range in the r - K spectrum. According to Anderson *et al.*, b for aphids should be highest, Noctuidae next and Geometridae lowest; birds should range most widely. In practice, Geometridae and Noctuidae are alike with the highest means, 2.22 and 2.25, Aphididae lower at 2.14, and birds lowest at 1.79; the range of b within groups does not notably differ⁷. Regressions (i)–(vi) (Fig. 1b) are from different generations in different population cycles from different sets of sites⁶ with no autocorrelation. According to propositions (1) and (2), each sample should fall on a different regression, yielding a very low correlation. In real samples of 444 species, more than 80% of the variance is accounted for in 80% of species and more than 90% in over half the species⁶.

(3) When species are sampled in two different sets of environments, b is constant but a may differ, as expected if environment affects variance in proportion to density (Fig. 2c). No uniform natural environment exists to test their proposition (3) but simulations of the Δ -model do generate linear log variance $\times$ log mean plots in uniform environments⁸.

(4) In published field data 0.4 (ref. 5) $< b < 3.9$ (ref. 7). Anderson *et al.*'s interpretation that "the vast majority of observed slopes lie between 1 and 2" (their Fig. 2 using our data) is taken from selected species, mainly pests⁵ or from temporal samples²⁹ where demographic models may have more relevance. However, they have overlooked our more extensive evidence in which 444, large-scale, consistent, unselected species' samples have $b > 2.0$ (Fig. 3), and some regressions are linear over four orders of magnitude of density^{6,7} (Fig. 1b). $b > 2$ implies individuals become more concentrated as mean density increases, as in active congregation. Theoretical premises¹, that immigration creates randomness and emigration creates 'under-dispersion' ($s^2 < m$), denote a fundamental misinterpretation of animal behaviour and migration¹². Demographic parameters do not account for active congregation and Anderson *et al.*'s simulations confirm this.

Δ -Model simulations yield regressions that are always linear with b ranging from 1.0 to >2.9 (ref. 17, Fig. 1c), closer to natural populations (Figs 1b, 3). The Δ -model introduced major

behavioural vectors into population dynamics, as Breder³⁰ did into fish schooling, by incorporating spatial dimensions into the dimensionless framework of theoretical models⁸. Its merit lies in a simple bifurcating rule for animal movement which simulates observed distributional relationships better than any other model. The Δ -model generates maps like geographical distributions of insect populations measured by synoptic monitoring^{8,17,31}. These maps resolve into hollow curves describing the frequency distribution of distances moved by actively migrating individuals to or from centres of concentration^{16,19}. The Δ -model predicts a power relationship between the mean and variance of the number of organisms per unit area with a species-specific component as found in field data¹⁷. It is, in effect, more parsimonious than Anderson *et al.*'s demographic model which has no less parameters but lacks parameters that reflect the species-specific components of spatial behaviour evident in common observation. These are a major topic of the study of animal social behaviour³². Population dynamic theory should be re-evaluated now that ease of computation diminishes the need for mathematical tractability. The Δ -model may not yet be definitive or adequate, but we prefer it to an inanimate principle that cannot express evolutionary mechanisms.

We do not see the Δ -model as conflicting with the foraging, feeding and reproduction modelling of Holling³³, Comins and Hassell³⁴, Krebs and Davies³², Maynard Smith³⁵ and others, or with optimization in social spacing mechanisms in birds and small mammals, but as complementing them. We merely extend the behavioural optimization approach to general spatial behaviour, the function of which is to optimize survival in the current and next generation by escape from competition and adversity, to find a better site in the real, fluid environment¹² in which static 'patches' are rare³⁶.

Migration is not regarded by us as being nonrandom solely because $b \neq 1$ but, *a priori*, on evolutionary grounds.

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Finding of a sex pheromone source by gypsy moths released in the field

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"Moths display some of the most striking examples of long-distance chemical communication in the animal kingdom"¹. Recent theories of how the male moths find a distant female have invoked steering by two different directional cues: (1) gradients of odour concentration in the air, used in 'aerial odour-trail following', and (2) the wind, used in 'odour-modulated anemotaxis'^{2–7}. Laboratory evidence has favoured the latter theory as well as contradicting the earlier radiation theories^{8–11}, but the issue could not be settled without recording in the field the flight track of a male moth and, simultaneously, the track of the odour plume. This has now been done with gypsy moths and wind-borne bubbles, confirming theory (2) and showing in addition that cross-wind casting itself contributes to a moth's progressive approach to the odour source, an unexpected consequence of the way odours are dispersed by the wind¹².

The observations were made over short grass in Silwood Park, Berkshire, at the first of two sites described previously¹², during July and August 1982 at air temperatures of 22–25 °C and wind speeds of 0–5 m s⁻¹. The wings of 3–5-day old gypsy moths, *Lymantria dispar* (L.), temporarily immobilized at 10 °C, were dusted with pink fluorescent powder to make them more visible. The moths were then re-caged and taken into the field where their flights were video-recorded from a 27-m-high tower as they passed through the experimental area at its foot. This area, 400 m², was marked in a grid every 2 m with numbered 1.2-m poles. Every other pole bore a pink paper streamer 30 cm long which clearly lifted whenever the wind speed exceeded about 0.4 m s⁻¹, each intervening pole bearing a light wind vane which showed the wind direction as long as the neighbouring streamers were lifted.

The attractant, 100 ng of the (+)-enantiomer of Disparlure¹³ evaporating from a 1-cm² square of filter paper, was placed near the upwind end of the area, 30 cm downwind of and 10 cm above the orifice of the bubble generator 1.3 m above the ground. The generator produced a stream of detergent bubbles each about 5 cm in diameter. Trials with titanium oxide smoke showed that the bubbles and smoke travelled together. Moths were exposed singly in an opened cage near the downwind end of the experimental area in or near the plume of bubbles, until they took off. Each moth was flown up to five times through the area, being recaptured with and re-released from a butterfly net. There was no apparent change in a moth's behaviour through such a series of releases. Other moths, used in an experiment to determine the effects of the bubbles on their flight, were flown as many times as possible alternately with and without the bubble generator in operation. This gave no indication that the bubbles had any effect on the behaviour of the moths, even when they were close to the source where the bubbles were densest. Nor did the wing dusting make any apparent difference to the moth's behaviour.

The video-records of the plume of bubbles agreed with a previous description of smoke plume behaviour¹², most bubbles being blown in fairly straight lines from the generator to the edge of the experimental area. When the plume of successive bubbles was bent, this was seldom due to bends in the tracks of individual bubbles. It was usually due to a change in the wind direction which could be seen progressing through the

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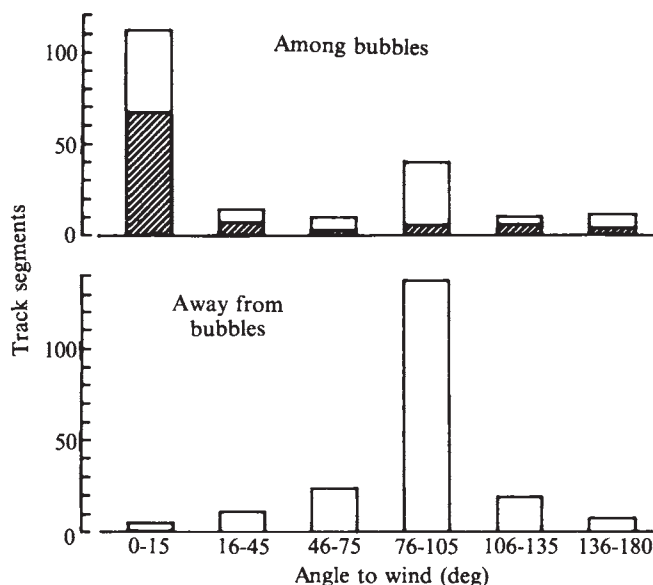


Fig. 1 Numbers of track segments at various angles to wind (due upwind = 0°), and numbers of these segments showing zigzagging (hatched), when moths appeared to be among the bubbles ($n = 199$) compared with when they were at least 1 m away from bubbles ($n = 201$). A 'track segment' is defined as a portion of flight track within 2 m of the ground that achieves a net horizontal displacement of two or more metres without deviations in excess of 0.5 m. 'Net displacement' refers to the resultant track during zigzagging, and to the actual track when not zigzagging. 'Zigzagging' is defined as two or more alternately left and right track excursions of 0.25–0.5 m and within 2 m of net displacement.

array of wind vanes and streamers, carrying bubbles away from the release point in the new direction while those released earlier continued in their original direction. On the days when the wind blew from the nearby tower and its associated huts towards the area, more bubble tracks were seen to be bent, presumably due to the extra turbulence induced by those structures.

Out of 31 moths that took off on release, 22 reached the odour source, doing so in 72 out of 130 flights. They flew mainly between 0.5 and 1.0 m above the ground, making much smaller vertical than horizontal excursions. Within the experimental area, a total of 62 flights of 6 or more metres were recorded from 19 moths, a total of 2,649 m of flight track in the horizontal plane. Of this distance, 63% (1,657 m) was classified, segment by segment, on the criteria given in the legend for Fig. 1, in terms of the measured angle between the direction of the moth's track and the direction of the wind at the same place and time. The directions of track segments among the bubbles (total 1,011 m) were then compared with those away from the bubbles (646 m) (Fig. 1). The remaining 37% (992 m) of track could not be classified in the same way because it consisted of portions that were too curved (total 606 m), too short (199 m) or too high (68 m) to meet the criteria for directional measurement, or because they occurred during wind lulls (61 m) or technical mishaps (59 m). This 37% of the flight track that is omitted from Fig. 1 contributed in all only 18% to the moths' progress towards the pheromone source.

When the moths appeared from high above to be among bubbles, then in reality they could have been above or below the bubbles and the pheromone plume, or within the plume 'envelope' but not in pheromone owing to the turbulent intrusion of clean air. Not surprisingly, therefore, there was a minority of cases (21%) when moths that appeared to be among bubbles behaved as they mostly did when well away from them, flying more or less across wind at 76–105° (Fig. 1). In most cases, however, moths apparently among bubbles flew on upwind tracks (56% at 0–15°) that varied from nearly straight to a series of sharp, narrow zigzags (Figs 1, 2). Zigzagging was observed only once when the moths were away from bubbles,

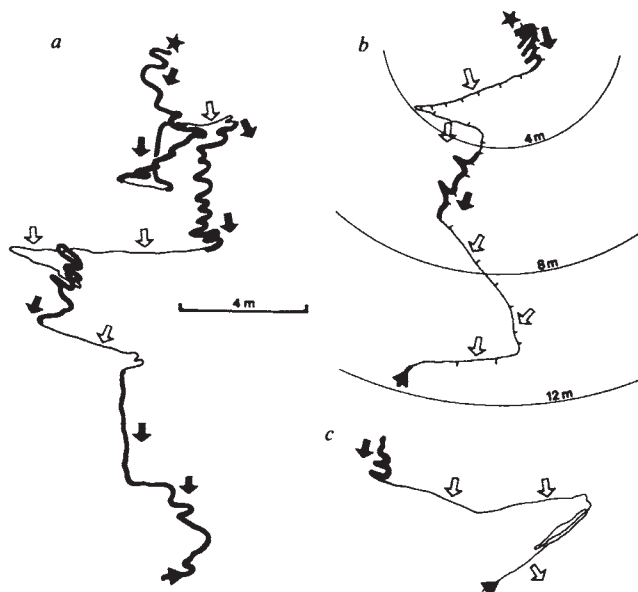


Fig. 2 Selected tracks of male gypsy moths approaching a source of both sex pheromone and bubbles (star). Thick line, track apparently among bubbles, with associated wind direction (solid arrows). Thin line, track when away from bubbles, with associated wind direction (open arrows). *a*, Progress towards source mainly by upwind flight among bubbles and pheromone (duration of track 50 s, wind speed when moth among bubbles 0.8–2 m s⁻¹); *b*, progress towards source mainly during cross-wind casting away from bubbles (time marks 0.5 s, duration 14 s, wind speed when moth among bubbles 1–3 m s⁻¹); *c*, portion of track 12 m from source showing change of direction with change of wind direction (wind speed when moth among bubbles 1.7 m s⁻¹).

whereas among the bubbles it occurred at all angles to wind (Fig. 1). It became more frequent as the moths neared the source, occurring in 51 out of 57 upwind track segments within 10 m of the source and in 14 out of 37 further out ($P < 0.01$) (see refs 8, 14, 15).

When a moth was flying among bubbles the pheromone source always lay within 15° of due upwind of the moth. Hence, on all of the 112 among-bubble track segments shown at 0–15° angles to the wind in Fig. 1, the moth was actually heading towards the source with not more than 15° error, confirming an earlier prediction¹².

When away from the bubbles the moths hardly ever advanced straight upwind and in 68% of cases flew to and fro across wind (Fig. 1) on wider excursions than when zigzagging, including long, almost straight casts (Fig. 2). Such casting across wind on losing the scent has been reported frequently from insects in wind tunnels^{2,5–7}. When the wind direction changed during a cross-wind cast, the moths changed direction until they were again flying across wind (Fig. 2c).

A moth often lost contact with the plume of bubbles because a change of wind direction had carried the next portion of the plume away from the moth. It then stopped advancing upwind and cast across the new wind direction, obliquely across the direct line from it to the source. Its casts therefore took it, alternately, further away from the source where it would not soon meet the diverted plume again, and nearer to the source where it was likely to do so. In consequence, when the casting moth re-entered the plume it usually did so at a point closer to the source than the point where the plume had been lost (Fig. 2b). Sometimes these oblique advances outside the plume contributed more to the moth's overall approach to the source, than did its upwind flight in the plume. Pooling the 62 flights, flight away from bubbles contributed 23% of all progress toward the source, while flight among bubbles contributed 59%.

These recordings showed that the moths approaching the pheromone source were steering by the wind, into it or across it according to its odour content: a case of odour-modulated

anemotaxis. This seems to rule out long-range steering by any kind of radiation from the source, or along the gradients of odour concentration in such a way as to follow the aerial odour trail laid by the shifting wind as a meandering plume. Had the moths been following the plume they would have been expected to turn aside with it when it came to lie across wind as a consequence of a wind direction change, but this was not observed. Their upwind zigzagging flight took them along the plume only when its axis was aligned on the source directly to windward. This upwind flight path took them out of the plume when it veered away but then they switched to wide casting to and fro across the new direction of the now odourless wind. Since this usually took them back into the plume again closer to the source than before, it was plainly a better tactic than following the drifting plume downwind, away from its source. Further measurements of the moths' behaviour will be presented elsewhere.

The moth pupae were generously supplied by the USDA Methods Development Centre at Otis Air Base, Massachusetts, and imported under licence no. PHF/29/58 from the Ministry of Agriculture. We thank Mr J. Ions for constructing the bubble generator, Mrs M. Jones for caring for the moths, Mrs E. Ludlow, Dr T. C. Baker and Ms G. Gibson for field assistance, Dr T. C. Baker for useful discussion, the Physics Department of Imperial College for the use of the tower, and Professor R. T. Cardé for the gift of pure pheromone. J.S.K. is a Senior Research Fellow at Imperial College.

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Possible relationship of morphogenesis in pathogenic fungus, *Histoplasma capsulatum*, to heat shock response

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Histoplasma capsulatum, like many other fungal pathogens, is dimorphic: it exists as mycelia in the soil and yeast in animal hosts. Because only the yeast phase is parasitic, factors which affect morphogenesis have been of interest for understanding and controlling pathogenicity. In culture, the mycelial to yeast transition of *H. capsulatum* is induced by a temperature shift from 25 to 37°C (ref. 1). The transition occurs over several days and is accompanied by marked changes in metabolic processes, including respiration and cysteine metabolism². Here, we show that the triggering event for these morphological and biochemical changes is a rapid decline in intracellular ATP levels that follows uncoupling of oxidative phosphorylation when mycelia are shifted from 25 to 37°C. We also show that respiration in the yeast phase is coupled at 37°C and thus that the morphological transition may be viewed as a heat shock followed by cellular adaptation to higher temperature.

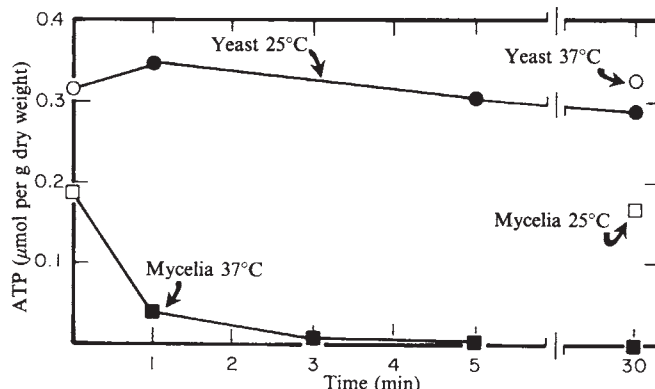


Fig. 1 Effect of temperature shifts on intracellular ATP levels. The Downs strain (mating type (–)) of *H. capsulatum* was grown to log phase in small (25 ml) cultures containing 2% glucose, 1% yeast extract². For temperature shifts, cultures were switched from one water bath at the starting temperature to another at the final temperature. In the controls, cultures were shifted from one water bath at the starting temperature to another at the same temperature. Cells were collected by rapid filtration and immediately frozen in liquid N₂. ATP was extracted with perchlorate¹⁰ and assayed by the glucose 6-phosphate dehydrogenase/hexokinase method¹¹. Each assay used 10–15 mg dry weight of cells. Values are expressed as μmol ATP per g dry weight. The experiment was carried out with three independent cultures and essentially identical results were obtained. □, Control, mycelia kept at 25°C; ■, mycelia shifted from 25 to 37°C; ○, control, yeast kept at 37°C; ●, yeast shifted from 37 to 25°C.

We have previously distinguished three stages in the morphological transition of the *H. capsulatum* Downs strain based on analysis of biochemical changes². Stage 1, immediately following the temperature shift, is characterized by the rapid cessation of protein and RNA synthesis and a progressive decrease in cell respiration rate. After 24–40 h, the cells enter a dormant period (stage 2) which lasts 4–6 days. Stage 2 cells have grossly decreased concentrations of mitochondrial electron transport components. Stage 3 is characterized by increasing concentrations of cytochrome components, resumption of normal respiration, induction of the yeast phase-specific cysteine oxidase, and completion of the transition to yeast morphology².

Figure 1 shows intracellular ATP concentrations as a function of time following temperature shifts in mycelia and yeast. The striking finding is that mycelia shifted from 25 to 37°C undergo an immediate, rapid decline in ATP levels (half time <1 min). By 5 min, the ATP concentration had fallen below the limit of detection and remained so for at least 30 min, the last time point at which ATP was measured. Figure 1 also shows that yeast phase cells have rather higher intracellular ATP levels than do mycelia and that these levels are not affected by the temperature shift which initiates the reverse transition from yeast to mycelia.

Cell respiration experiments suggest that the temperature shift also results in uncoupling of oxidative phosphorylation (Fig. 2). Direct assay of oxidative phosphorylation would require isolation of coupled mitochondria which has not yet been accomplished for *H. capsulatum*. In the cell respiration experiments, coupling of oxidative phosphorylation is assayed indirectly by the ability of an uncoupler, carbonyl cyanide *m*-chlorophenylhydrazone (CI-CCP), to stimulate respiration in the presence of oligomycin, an inhibitor of ATP synthetase^{3,4}. Figure 2a–c shows the key results for mycelia at 25°C and following a shift in temperature to 37°C. At 25°C, addition of oligomycin results in a modest inhibition of respiration, suggesting that the rate was already close to state IV. Subsequent addition of uncoupler results in a nearly threefold increase in respiration rate in the experiment shown (Fig. 2a) and as much as a fivefold increase in other experiments (not shown). These results indicate that respiration in mycelia at 25°C is coupled to oxidative phosphorylation. By contrast, when mycelia are

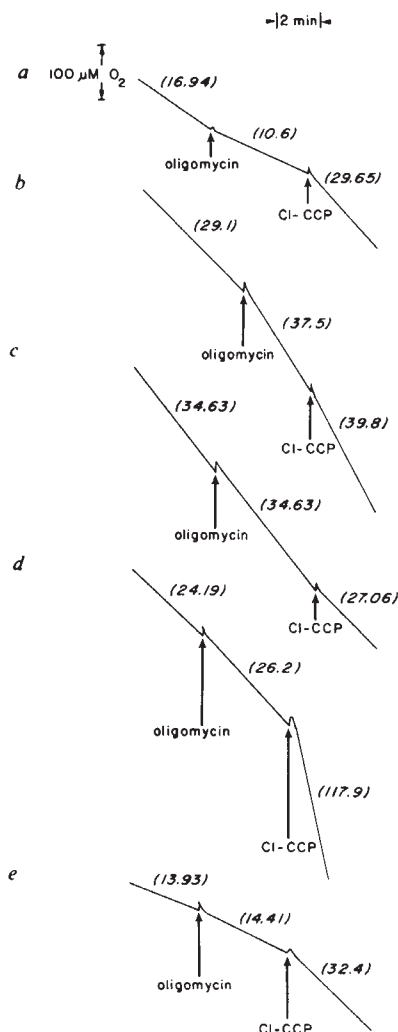


Fig. 2 Oxygen electrode recordings of respiration of mycelial and yeast phase cells. Cells (0.5 ml, 0.5–0.7 mg dry weight per ml) were resuspended in respiration buffer containing 1% mannose, 1 mM CaCl_2 and 1 mM dimethylglutaric acid (pH 7.2). Oxygen concentrations were measured polarographically using a KIC-oxygraph equipped with a Clark-type oxygen electrode (Gilson). The values in parentheses are rates of oxygen consumption (μl of O_2 per h per mg dry weight). Additions were oligomycin ($5 \mu\text{g ml}^{-1}$) and CI-CCP (0.1 mM) (both from Sigma). They were dissolved in absolute ethanol and were prepared freshly for each experiment. The experiment was repeated six times with independent cultures and similar results were obtained. *a*, Mycelia at 25°C; *b*, mycelia immediately after shift from 25 to 37°C; *c*, mycelia 5 min after shift from 25 to 37°C; *d*, yeast at 37°C; *e*, yeast immediately after shift from 37 to 25°C.

incubated at 25°C and then shifted to 37°C, there is an increase in respiration rate, no inhibition by oligomycin and no stimulation by subsequent addition of uncoupler (Fig. 2*b, c*). The establishment of this new pattern, indicative of uncoupled respiration, begins immediately after the temperature shift (Fig. 2*b*) and is complete by 5 min (Fig. 2*c*). By contrast, Fig. 2*d* and *e* show that respiration in yeast phase cells is tightly coupled at 37°C and remains coupled when cells are shifted to 25°C. The putatively uncoupled respiration in mycelia after the temperature shift is completely inhibited by cyanide plus salicylhydroxamic acid, which are inhibitors of cytochrome oxidase and alternate oxidase, respectively⁵.

The uncoupling of oxidative phosphorylation is sufficient to account for the rapid decline in intracellular ATP levels. However, other heat-induced damage may occur simultaneously and contribute to the decline. It seems likely that uncoupling simply reflects structural damage to the mitochondrial membranes caused by the temperature shift. In yeast, the

mitochondrial membranes may have undergone changes in lipid and/or protein compositions that allow respiration to remain coupled at 37°C. It is possible that parallel changes, for example, in lipid composition, occur in other cellular membranes and that these changes contribute to morphogenesis. We note that the data do not exclude more specific mechanisms of coupling and uncoupling.

The major metabolic changes that follow the temperature shift are readily explained by uncoupling of oxidative phosphorylation. The decline in ATP levels is sufficient to account for the rapid cessation of RNA and protein synthesis. The somewhat slower decline in cellular respiration rate was shown previously to be due to a progressive decrease in the concentrations of electron transport components⁵. The latter can in turn be attributed to cessation of protein synthesis combined with rapid turnover of mitochondrial inner membrane components (compare experiments with *Saccharomyces cerevisiae* which show that addition of uncoupler results in rapid disappearance of cytochrome components⁶). In the dormant stage, mitochondrial electron transport components are depleted and cysteine- or other sulphhydryl-containing compounds are required to reactivate mitochondrial respiration via shunt pathways that bypass blocked segments of the electron transport system⁵. The shunt pathways allow reoxidation of reducing equivalents and thereby increase the rate of substrate level ATP synthesis in glycolysis and the Krebs cycle. These findings, in conjunction with the present results showing that oxidative phosphorylation is uncoupled, readily explain the longstanding observation that cysteine is required at this stage to complete the morphological transition⁷. In stage 3 of the transition, resumption of normal respiration precedes the increases in RNA and protein synthesis by 1–2 days (unpublished results).

The Downs strain of *H. capsulatum* is an attenuated variety (50% lethal dose (LD_{50}) for mice, 2.0×10^7 organisms per ml) that is useful for routine laboratory purposes. Similar experiments were carried out on several virulent *H. capsulatum* strains (G184B, G186B and G217B; LD_{50} s for mice 5.0×10^5 – 4.2×10^6 organisms per ml) and on three other pathogenic fungi (*Candida albicans*, *Sporothrix schenckii* and *Blastomyces dermatitidis*) that undergo morphological transitions when the temperature is shifted from 25 to 37°C. In all these cases, the temperature shifts resulted in a transitory decline in ATP levels to about 50% of those of control cells. However, cell respiration remained coupled as judged by the effects of oligomycin and CI-CCP. The decline in ATP levels is consistent with the idea that a heat-related insult is common to the morphological transition of many or all dimorphic fungi. However, the insult in these other fungi seems to be less severe than that in the Downs strain of *H. capsulatum*. The prolonged dormant phase of the Downs strain presumably reflects the more severe insult and is not characteristic of more virulent *H. capsulatum* strains or of the other fungi tested (ref. 3, and unpublished material). The avirulence of the Downs strain may similarly be related to its greater sensitivity to temperature shifts.

Considered together, our results lead to the novel view that morphogenesis in pathogenic fungi may simply be a byproduct of the heat shock response which has been found in a wide variety of cell types and which has been extensively studied in *Drosophila*⁸. In other organisms, this stress response is characterized by induction of a small group of proteins, some of which are evolutionarily conserved⁹. Recent studies suggest that at least one of these conserved proteins is induced in *H. capsulatum* by the temperature from shift 25 to 37°C (unpublished). In *H. capsulatum*, changes in mitochondrial and cellular lipids may be part of the adaptation to higher temperature. These changes may provide new targets for controlling pathogenicity.

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Evidence for the T3-associated 90K heterodimer as the T-cell antigen receptor

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Several surface molecules appear to be involved in antigen recognition by human T lymphocytes including the monomorphic 20/25K T3 structure present on all mature T lymphocytes and the subset-specific associative recognition elements, T4 and T8 (refs 1-8). More recently, Ti_1 , a clonally unique antigen recognition structure comprised of a 49,000 molecular weight (49K) α -chain and a 43K β -chain, linked to T3 was identified on a major histocompatibility complex (MHC) class I specific $T8^+$ T-cell clone, CT8_{III} (ref. 9). To determine whether analogous receptor molecules could be found on other T-cell clones of differing specificity, we produced monoclonal antibodies against a clonal structure (Ti_2) on an MHC class II specific $T4^+$ lymphocyte, CT4_{II}, derived from the same donor as CT8_{III}. The Ti_2 structure on CT4_{II} is shown here to be a disulphide-linked heterodimer like Ti_1 on CT8_{III} and is composed of subunits of similar molecular weight. Monoclonal antibodies against Ti_2 or Ti_1 block antigen specific functions of the respective clone without showing any cross-reactivity. These findings suggest that each T lymphocyte, regardless of subset derivation or specificity, uses an analogous Ti heterodimer for antigen specific function. The latter is linked to T3 and expressed on the cell surface at an identical density (30,000-40,000 sites per cell).

Two monoclonal antibodies to a clonally unique recognition structure (Ti_1) have recently been produced by immunizing mice with an MHC class I specific human T-cell clone, CT8_{III}. The resulting antibodies were screened on the immunizing clone, and those reactive with CT8_{III} but lacking reactivity with additional clones from the same donor were selected. The anti- Ti_1 antibodies are unique in that they inhibit cell mediated killing and antigen specific proliferation of the immunizing CT8_{III} clone without affecting the function of any other clones tested⁹. Like anti-T3 antibodies, the anti-clonotypic antibodies enhance IL-2 responsiveness and induce modulation of the Ti_1 structure which co-modulates with T3. These studies indicate that the Ti_1 structure is closely associated with T3 in the membrane of CT8_{III}. Nevertheless, competitive binding and immunoprecipitation experiments using anti-clonotypic and anti-T3 antibodies demonstrate that Ti_1 is distinct from T3 and consists of two glycoprotein chains of 49K and 43K. In addition, Ti_1 is unrelated to the T8 surface structure⁹.

To determine whether the clonotypic structure on the MHC class II specific $T4^+$ T-cell clone CT4_{II} was similar to that identified on the class I specific $T8^+$ CT8_{III}, we developed a series of anti- Ti antibodies against CT4_{II} (ref. 10). Hybridoma cultures containing antibody reactive with CT4_{II} but unreactive

Table 1 Inhibitory effects of anti-clonotypic monoclonal antibodies on cytotoxicity of clones CT4_{II} and CT8_{III}

Medium	% specific lysis	
	CT4 _{II}	CT8 _{III}
Anti-T3 _A	56*	69
Anti-Ti _{1A}	9	22
Anti-Ti _{1B}	55	6
Anti-Ti _{2A}	56	17
Anti-Ti _{2B}	7	69
Anti-Ti _{2C}	6	68
Anti-Ti _{2C}	6	69

The human alloreactive T-cell clones CT4_{II} and CT8_{III} were derived by stimulating peripheral blood mononuclear cells of a healthy donor with the EBV transformed lymphoblastoid B-cell line Laz 156 as previously described⁴. Both clones have been propagated in culture for >21 months, maintaining stable phenotype and function^{2,4,9}. Briefly, CT4_{II}, derived from the $T4^+$ subpopulation of peripheral blood, was specifically directed at a class II MHC gene product expressed on Laz 156. In contrast, CT8_{III} was derived from the reciprocal $T8^+$ T-cell subset and directed at a class I MHC gene product on Laz 156. Here, CT4_{II} or CT8_{III} effector cells were incubated with one or another monoclonal antibody in ascites form at a final dilution of 1:500 or medium for 30 min at room temperature before addition of ⁵¹Cr-labelled Laz 156 target cells. The effector: target cell ratio was 30:1. The standard cytotoxicity assay was performed in V-bottomed microtitre plates (Falcon) for 4 h at 37 °C as described previously¹³. Standard derivations in the above experiments were <15%.

with both Laz 509, an autologous Epstein-Barr virus (EBV) transformed B lymphoblastoid line, and anti-T3 modulated CT4_{II} were selected, cloned and recloned by limiting dilution in the presence of feeder cells. Malignant ascites were then developed and used for analysis. Three individual monoclonal antibodies termed anti- Ti_{2A-C} were obtained from two fusions (3 of ~600 hybridomas). In contrast to anti-T3, these did not react with resting or activated human T cells or a series of additional T-cell clones ($n=25$) derived from the same individual, including CT8_{III}. Moreover, they were unreactive with B cells, monocytes, granulocytes or platelets from the donor of CT4_{II}.

To characterize the molecular nature of the Ti_2 structure on CT4_{II} and compare it with Ti_1 on CT8_{III}, solubilized membrane preparations were obtained from the externally ¹²⁵I-labelled CT4_{II} and CT8_{III} clones and antigens defined by anti- Ti antibodies precipitated and electrophoresed on SDS-polyacrylamide gels. As shown in Fig. 1, the molecule precipitated by anti- Ti_{2A} (and anti- Ti_{2B} and anti- Ti_{2C} ; not shown) from ¹²⁵I-labelled CT4_{II} cells appears as two bands and consists of a 51K α -chain and a 43K β -chain in reducing conditions (lane e). Moreover, in nonreducing conditions, this structure appears as a single band at ~90K (lane g). In contrast, anti- Ti_{2A} does not immunoprecipitate material from the ¹²⁵I-labelled CT8_{III} clone (lanes b, d). This is not surprising since anti- Ti_{2A} reacts with CT4_{II} but not with CT8_{III} by indirect immunofluorescence. In a reciprocal fashion, anti- Ti_{1B} precipitates material from ¹²⁵I-labelled CT8_{III} (lanes a, c), but not CT4_{II} (lanes f, h). The former appears as two bands of apparent molecular weight 49K and 43K on SDS-polyacrylamide gel electrophoresis (PAGE) in reducing conditions (a) and 90K in nonreducing conditions (c). Thus, although the Ti_2 and Ti_1 antigens are comparable in molecular characteristics and derived from T-cell clones of the same individual, they express unique structures which can be defined by non-cross-reactive monoclonal antibodies. The molecular basis for the slight difference in size and labelling intensities among the Ti α -chains of the two clones is still unclear. It is of interest that a similar molecular structure has now been identified by a monoclonal antibody to a murine antigen responsive T-T hybridoma¹¹.

Anti- Ti_2 antibodies affect cell mediated lympholysis and alloantigen induced clonal proliferation. As shown in Table 1, both CT4_{II} and CT8_{III} efficiently lyse ⁵¹Cr-labelled Laz 156 target cells (56% and 69%, respectively). In keeping with

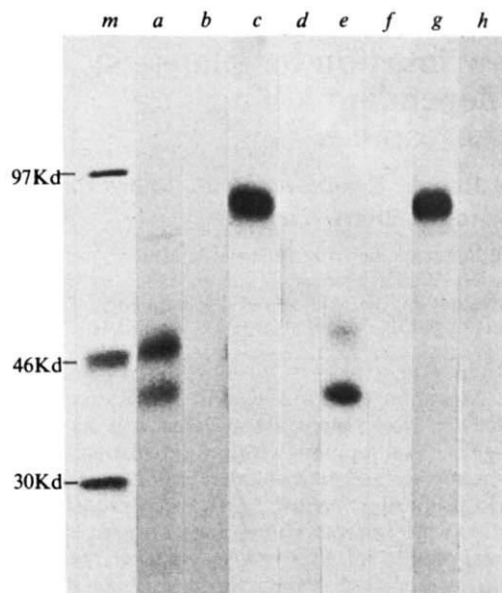


Fig. 1 Isolation and characterization of Ti_1 and Ti_2 molecules. 20×10^6 cells of either clone were surface-labelled with 1 mCi ^{125}I (NEN) using the lactoperoxidase method. Labelled cells were washed three times in phosphate-buffered saline and subsequently lysed in RIPA solution⁹. The lysates were then centrifuged at 10,000g for 10 min to remove nuclei and debris. After additional centrifugation at 100,000g for 30 min, the resulting supernatants were precleared in a two-step procedure using ImmunoPrecipitin (Bethesda Research Labs) for 30 min followed by monoclonal antibody T6 covalently linked to CnBr activated Sepharose 4B (Pharmacia) for 60 min at 4 °C. Immunoprecipitations were performed using the clonotypic antibodies anti- Ti_{1B} or anti- Ti_{2A} (both IgM) covalently linked to CnBr activated Sepharose 4B. Before electrophoresis, immunoprecipitates were washed three times with RIPA solution. SDS-PAGE was performed in reducing (R) and nonreducing (NR) conditions in a 12.5% polyacrylamide gel according to a modification of the Laemmli procedure¹⁷ followed by autoradiography. The following ^{14}C methylated molecular weight markers (NEN) were used (m): carbonic anhydrase (molecular weight 30,000); ovalbumin (46,000); phosphorylase b (97,000). Lanes a-d: CT8_{III}; lanes e-h: CT4_{II}. a, anti- Ti_{1B} (R); b, anti- Ti_{2A} (R); c, anti- Ti_{1B} (NR); d, anti- Ti_{2A} (NR); e, anti- Ti_{2A} (R); f, anti- Ti_{1B} (R); g, anti- Ti_{2A} (NR); h, anti- Ti_{1B} (NR).

previous findings, anti-T3 blocks the cytotoxic effector functions of both clones. In contrast, the anti-clonotypic antibodies anti- Ti_{2A-C} selectively inhibit killing by CT4_{II} ($\leq 7\%$) but not CT8_{III}, whereas anti-clonotypic antibodies anti- Ti_{1A-B} block killing by the CT8_{III} clone ($\leq 17\%$), but not CT4_{II}.

Table 2 indicates that CT4_{II} proliferates to the specific allogeneic B-cell line, Laz 156, as judged by increased 3H -thymidine incorporation (3,104 c.p.m. over basal level; 20 c.p.m.). Anti- Ti_{2A-C} or anti- Ti_{3A} antibodies markedly reduce this response (345–675 c.p.m.). In contrast, anti-I1 (ref. 12) antibodies reactive with human T-cell clones⁴ or monoclonal

Table 3 Quantitation of T-cell surface antigens

Cell	Surface molecule	P.Ch.Fl. (R.L.Fl.)	Binding sites per cell
CT4 _{II}	Ti_2	112 (900)	29,000
	T3	115 (950)	30,000
	T4	165 (3,700)	118,000
CT8 _{III}	Ti_1	126 (1,300)	42,000
	T3	130 (1,500)	48,000
	T8	180 (5,500)	175,000
T cells	T3	105 (780)	25,000
	T4	118 (960)	30,000
	T8	140 (1,900)	60,000

The number of binding sites for monoclonal antibodies was quantitated by indirect immunofluorescence using an Epics V cell sorter as well as ^{125}I -labelled monoclonal antibodies. Both methods gave identical results. All monoclonal antibodies with the exception of the anti-HLA (Sera Labs) w6/32 (IgG2) were of the IgG1 isotype and purified on protein A-Sepharose by affinity chromatography as described¹⁴. For radioimmunoassay, 3×10^5 CT4_{II} or CT8_{III} cells were incubated in triplicate with increasing concentrations of ^{125}I -monoclonal antibodies in the presence or absence of a 100-fold excess of the unlabelled ligand. Specific binding, the amount of ligand bound in ng at plateau values, was used to calculate the number of binding sites per cell. The variation among individual experiments was $\leq 15\%$. For analysis by flow cytometry, CT4_{II} and CT8_{III} cells were incubated with saturating concentrations of one or another monoclonal antibody and goat anti-mouse F(ab')₂ fluorescein isothiocyanate. Quantitation by this method is possible since fluorescence measured by a fluorescence activated cell sorter (FACS) is proportional with the number of bound fluorescein molecules per cell and is linear with the channel number using a linear amplifier¹⁵. The present quantitations are based on a valid calibration curve relating linear and logarithmic fluorescence on the Epics V cell sorter¹⁶. Since homogeneous fluorescence peaks were essentially gaussian on the log scale, their average fluorescence intensities (FI) were determined as the geometric mean (that is, the arithmetic mean channel number of log amplified intensities). For peripheral T cells, T4 and T8 fluorescence intensities were determined on the basis of the peak of reactive cells. The number of binding sites for monoclonal antibodies directed against Ia or HLA molecules with this analysis on both CT4_{II} and CT8_{III} was $\sim 20,000$ and $> 500,000$, respectively. Each T-cell population was analysed several times with consistent results (variation: $< 10\%$). P.Ch.Fl., peak channel fluorescence; R.L.Fl., relative linear fluorescence.

antibodies to the Ti_1 molecules, exclusively present on CT8_{III}, have no effect. Note, moreover, that anti- Ti_2 and anti- Ti_{3A} antibodies enhance the CT4_{II} clone's responsiveness to IL-2 but are not mitogenic by themselves. Given these findings, it is clear that the major blocking effects of anti-T3 or anti- Ti_1 antibodies are not due to a generalized diminution in cellular function. Moreover, these results are entirely analogous to those found on CT8_{III} cells using anti- Ti_{1A} , anti- Ti_{1B} and anti-T3 (ref. 9).

It is likely that the anti- Ti_1 and anti- Ti_2 anti-clonotypic antibodies define variable regions of the antigen receptors on CT8_{III}

Table 2 Influence of anti-clonotypic monoclonal antibodies on proliferative responses of clone CT4_{II}

Stimulus	Medium	Anti- Ti_{2A}	Anti- Ti_{2B}	Pretreatment Anti- Ti_{2C}	Anti- Ti_{1A}	Anti- Ti_{3A}	Anti-I1
Medium	20	18	22	65	34	119	13
Laz 156	3,104	345	373	675	3,200	415	2,958
IL-2	2,256	4,520	3,977	2,952	2,444	5,513	2,433

2.5×10^4 of CT4_{II} cells were incubated in round-bottomed microtitre wells (Costar) with 5,000 rad irradiated Laz 156 stimulator cells at a 1:1 ratio, or IL-2 containing supernatants⁴ (final concentrations 5%) at 37 °C in a humidified atmosphere containing 7% CO₂ in air. Monoclonal antibodies were used at a final dilution of 1:500 in medium and added at the start of the culture period. After 24 h, individual wells were pulsed with 0.2 μ Ci of tritiated thymidine (3H -TdR) (Schwarz-Mann) and collected 18 h later on a MASH II apparatus (M.J. Bioproducts, Walkersville). 3H TdR incorporation was then measured in a beta scintillation spectrometer (Packard Instruments). Each value represents the mean of triplicate samples. It should be noted that < 100 c.p.m. 3H TdR incorporation was obtained when CT4_{II} was cultured with irrelevant B lymphoblastoid cell lines.

and CT4_{II} respectively, since they recognize clonally unique epitopes and selectively inhibit the antigen specific functions of those individual clones. In contrast, the wide distribution of the 20/25K T3 glycoprotein and the ability of anti-T3 antibodies to inhibit antigen specific function of all clones suggest that T3 is a constant portion of the antigen receptor complex. The present findings support the view that the basic subunit composition of the antigen receptors on cells derived from both the T4⁺ and T8⁺ human T-cell subpopulations is similar. In contrast, recognition of class II or class I alloantigens by these subsets may be determined by the associative recognition structures T4 or T8 which are independent of Ti/T3 (ref. 9).

Given the strong evidence that the clonotypic structures are involved in antigen recognition and membrane associated with T3, it was important to quantitate the number of surface Ti and T3 molecules on CT4_{II}, CT8_{III} and resting peripheral blood T lymphocytes. Table 3 shows determinations of binding sites for monoclonal antibodies by quantitative fluorescence on an Epics V cell sorter. Comparable results were obtained using ¹²⁵I-labelled monoclonal antibodies (data not shown). Three points emerged from this analysis. First, the number of T3 and Ti molecules on each of the clonal populations is similar despite some interclonal variation in the absolute number of T3 and Ti molecules (CT4_{II} = 30,000 molecules; CT8_{III} = 42,000 molecules). This finding suggests a stochastic relationship in which one T3 molecule is linked to one Ti molecule in the cell membrane. Second, on the clonal populations, the density of the associative recognition structures T4 and T8 is far greater than T3/Ti (118,000 and 175,000 binding sites per cell, respectively). Third, in contrast to T3 (and presumably Ti) which is expressed to a similar degree on the resting T lymphocytes and T-cell clones, there are three- to fourfold fewer T4 and T8 molecules on resting lymphocytes of the appropriate subset derivation (30,000 and 60,000, respectively) than on the respective activated clonal populations.

T-cell activation may thus result in induction and expression of additional associative recognition structures, T4 and T8. This probably offers one explanation for their critical involvement in clonal effector function. In contrast, the number of antigen receptors defined by anti-T3/Ti remains comparable and hence fully expressed in the resting state before antigen binding.

The availability of the present set of monoclonal antibodies and human T-cell clones should make it possible to obtain precise biochemical data regarding the nature of these clonotypic, polymorphic and monomorphic structures at both the protein and DNA level. This, in turn, will help in the molecular understanding of T-cell activation and specificity.

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A new function for platelets: IgE-dependent killing of schistosomes

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Several killing mechanisms against schistosomes have been described *in vitro*, involving cellular and humoral factors. Neutrophils, eosinophils—with an accessory role for mast cells—monocytes and macrophages have been shown to exhibit cytotoxic properties against *Schistosoma mansoni* larvae, in association with antibodies of various isotypes or with complement (reviewed in ref. 1). Lymphocyte participation in effector functions is mediated mainly through lymphokines inducing cytotoxic macrophages², and, in certain cases, directly by T cells³. The experiments reported here show that platelets, taken from rats after specific periods of infection with *S. mansoni*, were able to kill schistosomes, and that normal human or rat platelets acquired toxic properties towards the same target in the presence of serum from infected individuals. The humoral factor involved in this process was shown to be IgE, and evidence was obtained of a Fc receptor for IgE on human and rat platelets. The passive transfer of immune platelets to normal rats conferred a high degree of protection towards a challenge infection by the parasite.

Platelets comprise nearly 5% of the total cell volume of the particulate elements of human blood, and 34% of the leukocyte volume. Apart from the lysis of antibody-coated erythrocytes⁴, platelets have not been described as cytotoxic effectors to pathogens. When incubated for 24 h or more with parasite larvae, platelets from normal Fischer rats showed no evidence of *in vitro* cytotoxicity against schistosome larvae in the presence of normal rat serum (20%) in Eagle's minimum essential medium (EMEM). During infection, however, platelets from *S. mansoni*-infected animals showed increasing killing properties, up to 98% at day 54, a period when rats expressed high levels of immunity to reinfection (Fig. 1). Similarly, the number of platelets in the blood of infected rats also increased up to three times the normal level at day 45 (Table 1). Both parameters then rapidly decreased after reaching the maximum value.

Once isolated from infected animals, the killing properties of immune platelets were only moderately influenced by humoral factors, as larval death was highly significant even with normal serum or with heat-inactivated fetal calf serum. Cytotoxicity fell by 5-10% in the presence of calf serum when compared with the immune platelets incubated with the autologous serum; intermediate results were obtained with normal serum.

It was therefore suspected that some factor capable of inducing platelet activity was present in the serum of infected individuals, and that platelets from normal subjects might be activated *in vitro* by the same sera. The simultaneous incubation of normal rat platelets with the serum of infected animals and schistosomes led to various degrees of killing according to the period of infection. A maximum was reached 42 days after the beginning of the disease in the rat (Table 2). Similarly, with human platelets, a high percentage of schistosome killing was observed in the presence of serum from patients with schistosomiasis. A short preincubation of the platelets before the addition of the target larvae gave even higher schistosomicidal effects.

Experiments carried out to characterize the humoral factor(s) involved in this process showed it to be heat-labile and not

Table 1 Blood platelets in rat schistosomiasis

Normal rats		221,500 ± 93,742
Infected rats	Day 3	281,400 ± 78,730
	Day 26	627,750 ± 107,121
	Day 31	705,250 ± 237,770
	Day 42	755,600 ± 123,417
	Day 52	538,000 ± 125,310

Blood platelets were counted in a haemocytometer under phase contrast microscopy after retro-orbital puncture, and dilution in London staining mixture. Results are the mean ± s.d. per mm³ of eight rats infected with 1,500 *S. mansoni* cercariae.

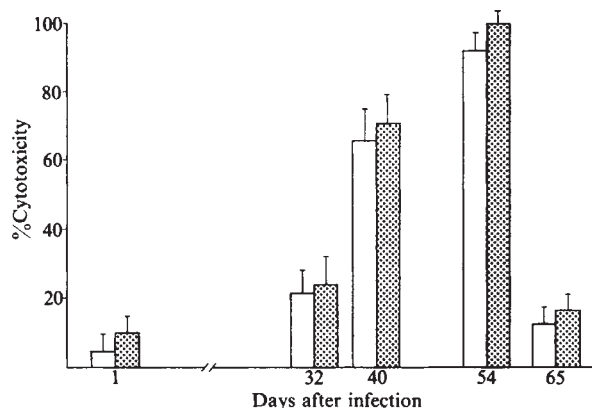


Fig. 1 Killing properties of rat platelets against schistosomula at different periods of *S. mansoni* infection. 2 ml of heparinized blood from the retro-orbital sinus were diluted with 18 ml heparinized EMEM and centrifuged at 400g for 15 min at 4 °C. Platelets in the supernatant were washed with heparinized EMEM by centrifugation at 5,000g for 20 min at 4 °C. 7.5×10^7 platelets in 0.1 ml EMEM were dispensed into flat-bottomed microplates, with 20 µl normal or infected rat serum and 50 schistosomula in 80 µl EMEM. After 24 h at 37 °C and 5% CO₂, motionless and dark dead larvae were clearly distinguished from mobile and refringent living schistosomula by optical microscopy. Open bars, platelets in the presence of normal rat serum; shaded bars, platelets in the presence of infected rat serum. Mean ± s.d. of six experiments in duplicate.

Table 2 Serum-induced anti-schistosome properties of rat and human normal platelets

	% Dead schistosomula	
	With platelets	Without platelets
Normal rat serum	13.8 ± 5.9	6.1 ± 3.8
Infected rat serum		
Day 35	28.5 ± 6.4	7.5 ± 3.0
Day 40	61.0 ± 11.7	10.3 ± 5.0
Day 42	81.3 ± 9.1	14.8 ± 2.2
Day 49	58.5 ± 20.5	5.5 ± 4.5
Day 54	19.0 ± 8.5	8.5 ± 3.5
Day 77	16.0 ± 4.2	14.5 ± 1.5
Normal unheated serum + day 42 heated serum	8.3 ± 5.5	7.6 ± 3.1
IgE-depleted rat serum		
Day 42	24.3 ± 3.8	8.5 ± 2.1
IgG-depleted rat serum		
Day 42	83.3 ± 8.4	3.0 ± 1.4
Aggr. IgE + infected rat serum		
Day 42	28.3 ± 2.8	10.0 ± 2.3
Aggr. IgG + infected rat serum		
Day 42	67.9 ± 5.9	12.0 ± 2.1
Normal human serum		
Without preincubation	16.8 ± 9.7	5.7 ± 2.1
With 1 h preincubation	16.0 ± 9.8	—
With 5 h preincubation	19.3 ± 5.0	—
Serum from patients with bilharziasis		
Without preincubation	68.5 ± 5.3	8.3 ± 2.6
With 1 h preincubation	88.0 ± 5.3	—
With 5 h preincubation	76.0 ± 7.4	—
Heated + normal serum	25.3 ± 3.8	6.5 ± 3.2

Blood platelets from uninfected individuals were prepared as described in Fig. 1 legend, and incubated in flat-bottomed microplates at 37 °C in 5% CO₂, with 20% rat or human serum and 50 schistosomula in EMEM. The percentage of dead larvae was evaluated after 24 h. The IgE level in the serum from 42-day infected rats was 6.0 µg ml⁻¹ before immune adsorption and undetectable after that; the IgG level was 9.5 mg ml⁻¹ before and 2.3 mg ml⁻¹ after immune adsorption. Heat inactivation of the serum from infected subjects was performed at 56 °C for 3 h and complement restored with normal serum. Rat monoclonal IgE was aggregated (aggr.) with dimethyl suberimidate and used at the concentration of 10 µg ml⁻¹; IgG was aggregated by heating for 20 min at 63 °C and used at 5 mg ml⁻¹.

Table 3 Transfer of immunity to normal syngeneic rats with immune platelets

	Mature worms	Immature worms	% Immature worms	Total worms	% Protection
Normal platelets	119.3 ± 27.6	44.8 ± 13.9	27	164.0 ± 36.7	—
Day 46 immune platelets	35.5 ± 23.8	24.8 ± 13.8	41	60.3 ± 35.7*	63.3 ± 23.3
Day 67 immune platelets	63.5 ± 31.6	39.8 ± 22.7	38	103.3 ± 54.3	35.5 ± 35.9

Uninfected female Fischer rats (200 g) were injected intravenously with 1.4×10^9 platelets 1 h before infection by percutaneous exposure to 1,000 *S. mansoni* cercariae. Liver perfusion for worm enumeration was performed 21 days later. Results are the mean ± S.D. of four rats in each group.

* $P < 0.005$.

restored by fresh unheated normal serum. Therefore, complement could be excluded, at least as an essential parameter for the induction of killer platelets (Table 2). In fact, solid-phase immunoabsorption of IgE, and not of IgG, from the serum of 42-day-infected rats, abrogated its ability to promote toxic properties in normal platelets. Furthermore, unrelated monoclonal IgE, but not IgG, inhibited the immune serum-induced platelet toxicity, providing further evidence of a crucial role for IgE in platelet activation. Preliminary experiments with heterologous parasitic targets indicate that this killing process is specific for the parasite of the donor's infection.

Finally, the passive transfer of immune platelets from 42-day-infected rats to normal syngeneic recipients on the day of a challenge infection led to a high degree of protection, with a 63% reduction in the total number of worms in the liver 3 weeks after infection, when compared with control rats injected

with the same number of normal platelets (Table 3). A concomitant increase in the number of immature worms was observed in animals receiving immune platelets, even from day 67, a period when immunity declines.

The present results provide the first demonstration of a direct effect of platelets on pathogens, not only in a cytotoxic process *in vitro*, but also as inducers of a significant degree of protection to a challenge infection when transferred from immune to naive animals. Bearing in mind the effector mechanisms already described *in vitro* against schistosomes, this killing process must be integrated into the other lethal activities of the immune system towards parasites, and, above all, must take into account the efficient escape mechanisms developed by schistosomes to balance their apparently very high probability of death in immune animals¹. Although it might seem like one more twist in the saga of *in vitro* killing reactions against schistosomes,

this new mechanism allows us to propose the unifying concept that most of these processes rely on the interaction of IgE antibody with low-affinity receptors on various cell populations (macrophages, eosinophils, and now platelets).

The observation that IgE interacted directly with platelets—probably as schistosome-specific antibody, since nonspecific rat myeloma IgE did not induce platelet cytotoxicity—indicates that the participation of blood platelets deserves consideration in various immunological situations in which only mast cells or basophils have previously been implicated. Receptors for IgG have been described^{5,6}, and IgG-dependent lytic properties against sheep red blood cells have been observed⁴. The data reported here concern the problem of a receptor for IgE on platelets: the inhibition of the immune serum-induced platelet toxicity by unrelated myeloma IgE, and not IgG, is the first evidence of such a receptor on these blood elements.

On the other hand, when isolated from immune animals, platelets showed very high levels of cytotoxicity against schistosomula, even in complement-free medium such as EMEM containing 20% fetal calf serum. Taken together with the passive transfer of immunity by platelets from infected animals and with the abolition of immune serum effects on normal platelets by IgE adsorption, the present findings of an interaction between IgE and platelets extend the known properties of blood platelets to effector functions. The precise analysis of mediators of their adherence and cytotoxic activities should shed light on the participation of these blood elements in cell immunity and cooperation, as well as in the understanding of several immunopathological reactions.

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Functional V region formation during *in vitro* culture of a murine immature B precursor cell line

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B lymphocytes originate from pluripotential haematopoietic stem cells and differentiate into immunoglobulin (Ig)-producing cells. B-cell lineage differentiation is accompanied by two types of immunoglobulin gene rearrangements^{1–3}—rearrangement of V, D and J gene segments to create a functional V gene and rearrangement of C_H genes for heavy-chain switching. These results, however, have been obtained mainly by analysis of immunoglobulin gene organization of myeloma cells. Baltimore and his colleagues have established Abelson murine leukaemia virus (A-MuLV)-transformed cell lines⁴ and found a few lines capable of carrying out κ -gene rearrangement⁵ or undergoing isotype switching during *in vitro* culture⁶. To study early B-cell lineage differentiation events, we have now also established A-MuLV-transformed cell lines which are capable of

differentiating from μ^- to μ^+ and of undergoing continuing rearrangement of heavy-chain genes in culture. Analysis of immunoglobulin gene organization of these transformed cells revealed that μ^- cells have already undergone DNA rearrangements involving J_H segments but an additional rearrangement of J_H segments is required for initiation of μ -chain synthesis. Southern blot analysis of the DNA and two-dimensional gel electrophoresis of intracytoplasmic μ -chain show that μ -chain diversity with respect to antigen specificity may be generated during this second rearrangement process. As no rearrangement of light-chain genes takes place in these cells, this implies that light-chain gene rearrangement requires some further change, or a different enzyme.

AT11-2, which was an A-MuLV-transformed cell line derived from BALB/c mice and designated B2 in a previous paper⁷, included 0.1–0.2% of intracytoplasmic μ -positive (μ^+) cells when tested by immunofluorescence. Of 57 subclones which had been isolated by cloning of AT11-2 cells in 0.3% soft agarose, 51 also included 0.01–0.43% of μ^+ cells, suggesting that μ^+ cells were being generated from μ^- cells during culture. We attempted to select μ^+ cells by further cloning of subclones and isolated secondary, tertiary and quaternary subclones in which 100% of cells had intracytoplasmic μ -chains (Fig. 1). We also isolated several μ^- subclones, which like the parental line included only 0.3–0.5% μ^+ cells. In μ^+ subclones, cells synthesized μ -chains, but not heavy chains of other isotypes nor κ - or λ -chains, when tested by immunofluorescence. To confirm the synthesis of μ -chains, cells were pulse-labelled with ³⁵S-methionine and newly synthesized immunoglobulins analysed by SDS-polyacrylamide gel electrophoresis. All μ^+ subclones synthesized μ -chains but not light chains (data not shown). All μ^+ and μ^- subclones had κ -chain genes of both chromosomes in germ-line configuration, when BamHI/EcoRI-digested DNAs were hybridized with the 3.9-kilobase (kb) HindIII/BamHI fragment containing C_κ gene as a probe. Therefore, immunoglobulin light-chain genes were neither rearranged nor expressed in any of the lines.

The configuration of immunoglobulin heavy-chain genes was studied in five μ^+ and eight μ^- subclones by Southern blot analysis. The DNA was digested with EcoRI or XbaI and fragments were hybridized with the 1.5-kb J_{H4} HindIII/EcoRI probe previously described⁷ and indicated in Fig. 2a. EcoRI restriction fragments of all μ^- subclones gave 11.0- and 5.4-kb bands as did the parental line and these differed from the single 6.6-kb band obtained from somatic (liver) DNA (Fig. 2b). Similarly, the parental line DNA was not distinguishable from that of any of the μ^- clones in terms of XbaI fragments (Fig. 2c). XbaI cuts within the DNA complementary to our J_H probe and as expected, liver yielded two fragments of 0.9 and 3.8 kb. Rearrangements involving any one of the four J_H segments would alter the size of the 3.8-kb fragment. As shown in Fig. 2c, DNA from all the μ^- lines as well as the parental line gave new XbaI fragments of 5.6 kb. Comparison of the band intensity among DNAs from μ^- and μ^+ clones as well as the data from the EcoRI digestion indicate that this 5.6-kb band must be produced by the migration of two bands to the same position. We conclude from these results that J_H rearrangements had occurred in both heavy-chain alleles of the parental μ^- line, and μ^- subclones did not undergo further rearrangements during subsequent cloning and propagation.

A quite different picture emerged from the analysis of immunoglobulin genes in the five μ -chain-synthesizing clones. All of these had apparently undergone a second rearrangement of at least one of the IgH alleles and this was apparent with both EcoRI and XbaI digestion. When digested with EcoRI (Fig. 2b), μ^+ subclones, AT69 and AT51-2-1, yielded a new fragment of 3.3 and 1.6 kb, respectively, instead of the 11.0-kb fragment seen among all μ^- subclones, but shared the 5.4-kb EcoRI fragment with μ^- subclones. An XbaI digest of AT69 and AT51-2-1 DNAs revealed a new fragment of 6.0 and 9.4 kb, respectively, and shared two XbaI fragments of 5.6 and 0.9 kb with μ^- subclones, although the intensity of the 5.6-kb

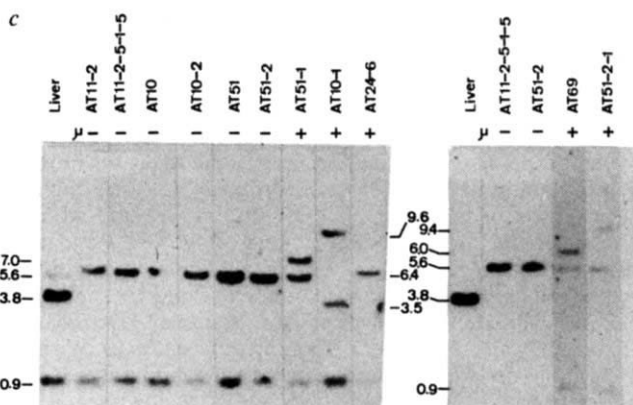


Fig. 2 Analysis of heavy-chain gene rearrangements in intracytoplasmic μ^- and μ^+ subclones. *a*, Restriction map of J_H - C_H region. Relevant *EcoRI* (E) *XbaI* (X) and *HindIII* (H) sites are indicated. *b*, DNAs digested with restriction enzyme *EcoRI* were electrophoresed in 0.5% agarose gels, transferred to nitrocellulose filters and hybridized with a 1.5-kb J_{H4} *HindIII*/*EcoRI* probe described previously⁷. Hybridization was performed at 65°C in 1 M NaCl, 50 mM Tris-HCl (pH 7.4), 10 mM EDTA, 0.2% bovine serum albumin, 0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.1% SDS and 100 μ g ml⁻¹ sonicated denatured *Escherichia coli* DNA. *c*, DNAs were digested with restriction enzyme *XbaI* and Southern gel blotting analysis was performed as described in *b*. The captions to subclones are abbreviated as described in Fig. 1 legend.

tested (AT51-1, AT10-1, AT24-6), further rearrangements seem to have occurred upstream of J_3 , although further rearrangement on another allele of AT10-1 eliminated the J_3 gene. Hybridization of *Eco*RI-digested DNAs from a μ^- clone with the C_μ probe revealed two bands of 12.5 and 11 kb, suggesting the partial deletion within the 12.5-kb C_μ -bearing *Eco*RI fragment on one allele. This configuration remained unaltered in μ^+ clones (data not shown). All of these results showed that the recombination sites in the doubly rearranged alleles in μ^+ clones were on the 5' side of the J_H region.

The finding that a second DNA rearrangement has occurred in all of the lines that synthesize immunoglobulin raises two important questions. First, which of the two discrete rearrangement events involves *V* gene selection, that is, which is responsible for the generation of antibody diversity? The variability in *J_H*-containing fragment size in each of the μ^+ subclones and the double digestion of DNAs suggested that the second

Fig. 1 Isolation of intracytoplasmic μ^+ and μ^- subclones by repetitive recloning of a μ^- parental clone (AT11-2). An A-MuLV-transformed cell line, AT11-2, was established by *in vivo* transformation as described previously⁷. Briefly, the stock of A-MuLV was injected intraperitoneally into newborn BALB/c mice. One month later, cell suspensions were prepared from enlarged spleens, cultured in RPMI 1640 medium with 20% fetal calf serum (FCS) and cloned in 0.3% agarose medium. Repetitive cloning was carried out in 0.3% soft agarose containing RPMI 1640 medium with 10% FCS and 5×10^{-5} M 2-mercaptoethanol. AT11-2-5-1-5, an intracytoplasmic μ^- subclone, was obtained after recloning AT11-2 three times. AT11-2-5-1-5 was further recloned and three representative clones, a μ^+ clone, AT69, and μ^- clones AT10 and AT51, were isolated. AT10 and AT51 were further recloned and μ^+ or μ^- subclones were isolated. Because each μ^+ subclone showed a different pattern in the rearrangement of the heavy-chain genes or in two-dimensional gel electrophoresis of newly synthesized μ -chains, they have been represented by different shapes, for example, $\square^+$, $\triangle^+$, $\square^-$, $\triangle^-$.

*Xba*I fragment was markedly reduced in these μ^- subclones (Fig. 2c). In AT51-1 subclones, an *Eco*RI digest revealed a single 5.4-kb fragment and the *Xba*I digest yielded a new *Xba*I fragment of 7.0 kb in addition to 5.6- and 0.9-kb fragments seen in all μ^- subclones, indicating that two *Eco*RI fragments co-migrated to the same 5.4-kb position. These observations show that in these three clones, AT69, AT51-2-1 and AT51-1, further rearrangement within or near the J_H segment and also upstream from the 3' *Xba*I site of the J_{H4} gene has occurred on one allele, whereas the other allele remains unaltered. A μ^+ subclone, AT10-1, yielded two *Eco*RI fragments of 6.8 and 2.5 kb and did not share any fragments with μ^- subclones (Fig. 2b). *Xba*I digestion produced two new fragments of 9.6 and 3.5 kb instead of the 5.6-kb fragment seen in all μ^- subclones (Fig. 2c). The result indicates that further rearrangement of the J_H segment has occurred on both alleles in AT10-1. AT24-6 produced a new single *Eco*RI fragment of 4.5 kb and lost the two fragments of 11.0 and 5.4 kb seen in all μ^- subclones (Fig. 2b). *Xba*I digestion also revealed a new fragment of 6.4 kb and the loss of the 5.6-kb fragment (Fig. 2c), indicating that further rearrangement of the J_H segment has occurred on one allele and that the other allele has been deleted. To map the recombination sites in the doubly rearranged alleles, further enzyme analysis was carried out among DNAs from μ^- and μ^+ clones. As germ-line DNA bears a *Bgl*II site between J_1 and J_2 , a *Bam*HI site between J_2 and J_3 , a *Pst*I site in J_3 and a *Hind*III site between J_3 and J_4 , double digestions (*Bgl*II/*Eco*RI, *Bam*HI/*Eco*RI or *Hind*III/*Eco*RI) and *Pst*I digestion would allow us to map the recombination sites in μ^- and μ^+ clones. As shown in Fig. 3, in a μ^- clone, AT11-2, recombination has apparently occurred with J_1 on both alleles. In three μ^+ clones

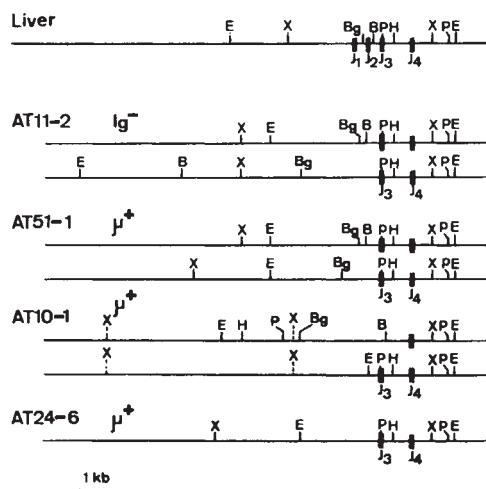


Fig. 3 Restriction map comparisons of the J_H segments in AT11-2 (parent μ^- clone) and AT10-1, AT51-1, AT24-6 (μ^+ subclones). These restriction endonuclease cleavage sites were identified by Southern blotting experiments. DNAs were digested with *EcoRI*, *HindIII*, *PstI*, *XbaI*, *BglII/EcoRI*, *BamHI/EcoRI*, or *HindIII/EcoRI* and hybridized with the J_H probe. As it is not clear to which allele a *XbaI* site in AT51-1 belongs, both sites are indicated by broken lines.

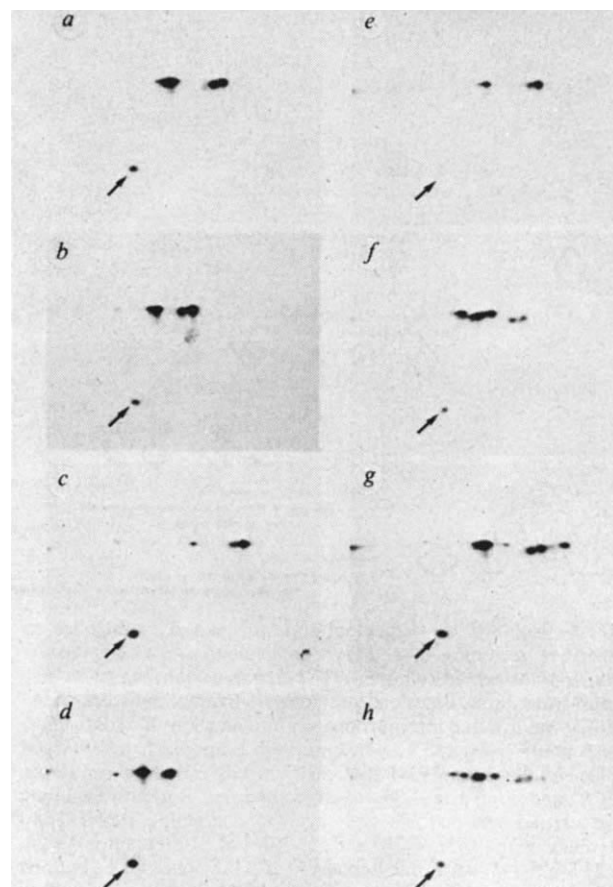


Fig. 4 Two-dimensional gel analysis of μ -chain molecules synthesized by intracytoplasmic μ^+ subclones. *a*, AT10-1; *b*, AT69; *c*, AT24-6; *d*, AT51-2-1; *e*, AT51-1; *f*, mixture of AT10-1 and AT69; *g*, mixture of AT10-1 and AT24-6; *h*, mixture of AT10-1, AT69, AT24-6, AT51-2-1 and AT51-1. The arrows indicate a spot of actin which was precipitated nonspecifically and can be used as an electrophoretic marker to compare different gels. All gels were shown with the origin of the first dimension (the acid end) on the left and decreasing molecular weight from top to bottom. Cells were incubated in methionine-free RPMI 1640 medium containing 10% dialysed FCS and ^{35}S -methionine (200 Ci mmol^{-1} , NEN) for 3 h and lysed. Labelled μ -chains were immunoprecipitated with rabbit anti-mouse μ -chain serum and *Staphylococcus aureus* Cowan I and analysed on two-dimensional gels with a non-equilibrated pH gradient in the first dimension and 9% SDS-polyacrylamide gel in the second dimension.

recombination event may involve V - D joining and be responsible for the generation of antibody diversity. We therefore used isoelectric points of the synthesized μ -chains as an index of their variability (Fig. 4). Two-dimensional gels were run with the products of all five μ^+ clones (Fig. 4*a-e*) and with the mixture of the products of μ^+ clones (Fig. 4*f-h*) and eight patterns are shown in Fig. 4. All the μ -chains were approximately the same size, but we cannot exclude the possibility that postsynthetic modification (for example, glycosylation) differentially affects μ -chains from the different lines. With this reservation, no two μ -chains were the same and this would be consistent with each utilizing a different V region gene.

A second question raised by this study is whether the second rearrangement step is obligatory for transcription of a functional μ -chain messenger. In studies to be reported elsewhere, we obtained evidence that this may be the case. Fusions were done between the μ^+ clone, AT69, and a non-immunoglobulin-secreting myeloma line (P_3U_1) which was a mutant from X63 γ_1 myeloma cells. Most of the hybrid progeny were unstable and the percentage of μ -producing cells in several clones decreased during *in vitro* culture when examined by immunofluorescence. DNAs from μ^+ and μ^- hybridomas were digested with *EcoRI* and hybridized to the J_H probe. Clones containing 100% μ -producing cells revealed three fragments of 6.6, 5.4 and 3.3 kb of which the latter two fragments were derived from AT69. However, the 3.3-kb fragment was not detected in μ -negative hybrid clones, showing that the doubly rearranged chromosomes were expressed (data not shown).

The present study demonstrates the spontaneous differentiation of an A-MuLV-transformed cell line (AT11-2) from μ^- to μ^+ stage while growing in culture. The differentiation from μ^- to μ^+ stage is accompanied by the further rearrangements of heavy-chain genes including J_H segments. Similar continuing rearrangement of κ -chain genes was also demonstrated in another A-MuLV-transformed cell line^{5,8}. Thus, AT11-2 cells may not represent a special case and other A-MuLV-transformed cell lines may retain the capacity to carry out various sequential rearrangements of immunoglobulin genes *in vitro*. The clone used in the present study may be comparable with normal early B-cell precursors because: (1) J_H rearrangement has already occurred on both alleles⁹; (2) μ -chain synthesis is not observed¹⁰; (3) κ -chain genes are in the embryonic configuration^{4,11-13}; (4) μ^+ subclones, in which κ genes are still

in the embryonic configuration, emerge spontaneously from μ^- AT11-2 cells.

Several previous observations lead us to think that the J_H rearrangements observed in lines like AT11-2 represent D_H - J_H joining. D_H - J_H joinings are often found in the unexpressed copy of chromosome 12 in myelomas¹⁴. Human leukaemic B cells were also shown to have one complete V_H - D_H - J_H recombinant and one D_H - J_H type intermediate¹⁵. Rearrangements observed in T-cell clones are also D_H - J_H joining¹⁶. These results suggest that D_H - J_H joinings may precede V_H - D_H - J_H rearrangements, and early B-cell precursors, typified by AT11-2, may contain D_H - J_H joinings on both chromosomes. The next recombinational event may involve selection of a single V_H out of some hundreds of V_H segments and bringing it into proximity with the D_H - J_H segment. This process could make it possible to initiate expression of μ -chains. In several cell lines examined (AT69, AT51-2-1 and AT51-1), the secondary rearrangement occurred on only one allele. This could be used to explain the mechanism of 'allelic exclusion'. Cloning and sequencing of rearranged genes in AT11-2 and μ^+ subclones derived from AT11-2 should provide insight into the sequential events required for the creation of functional V genes.

As demonstrated in AT11-2 cells, continuing rearrangements have occurred in heavy-chain genes during culture, but κ -chain genes stay in the germ-line configuration in secondary, tertiary and quaternary subclones. Therefore, the enzyme or process involved in the rearrangement of heavy-chain genes may not be capable of inducing the rearrangement of κ -chain genes.

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HLA-D region β -chain DNA endonuclease fragments differ between HLA-DR identical healthy and insulin-dependent diabetic individuals

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The human *HLA-D* histocompatibility region encodes class II antigens each of which consists of two polypeptide chains (α and β) inserted in the plasma membrane. These molecules are implicated in the regulation of the immune response¹⁻³ but several human diseases are also found to be associated with certain HLA-DR antigens⁴. The occurrence of insulin-dependent (type I) diabetes (IDDM) is strongly associated with HLA-DR3 and/or 4 (ref. 5). The class II antigens, however, show a marked genetic polymorphism¹ associated with the β -chains^{6,7} which seem, from hybridization studies, to be encoded by several genes^{8,9}. We have therefore used the β -chain cDNA probe, pDR- β -1 (refs 8, 10) to test whether there are differences in hybridization pattern between DNA from healthy individuals and diabetic patients, after digestion with restriction endonucleases. Among the HLA-DR 4 and 3/4 individuals, the IDDM patients showed an increased frequency of a *Pst*I 18 kilobase (kb) fragment. A *Bam*HI 3.7 kb fragment, frequent among controls (30-40%), was rarely detected in the IDDM patients (0-2%). These differences may be related to susceptibility to develop the disease.

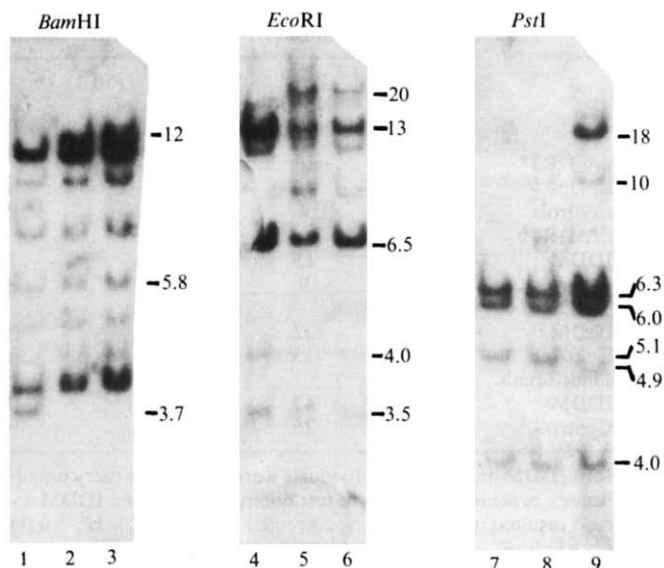


Fig. 1 *Bam*HI (1-3), *Eco*RI (4-6) and *Pst*I (7-9) genomic DNA restriction fragments obtained by hybridization to HLA-D region β -chain cDNA sequences^{8,10,21}. Lymphocytes from HLA-DR3/4 healthy individuals JH (lanes 1, 4, 6), BLP (2, 5, 8) and JF (3, 6, 9) were prepared as previously described¹².

Methods: DNA was isolated by solubilizing the cells at 37 °C for 16 h in 10 mM Tris (pH 8.0), 2 mM EDTA, 10 mM NaCl, 1% SDS and 0.2 mg ml⁻¹ proteinase K. Protein was removed from high molecular weight DNA by phenol-chloroform-isoamyl alcohol extraction and the DNA was recovered by ethanol precipitation. Samples of 5 μ g DNA were digested for 15-20 h in a final volume of 100 μ l with 24 units of *Bam*HI, 22 units of *Eco*RI or 12.5 units of *Pst*I according to the manufacturer's (Boehringer) specifications. The digested DNA samples were electrophoresed overnight on 1.0% agarose gels in TEAC (40 mM Tris-acetate, pH 8.0, 2 mM EDTA) at 25 V (*Eco*RI) or 30 V (*Bam*HI, *Pst*I). Fragments of *Hind*III-digested DNA (Bethesda Labs) were used as molecular size markers. The DNA in the gels was transferred to nitrocellulose sheets (Schleicher & Schuell) by the method of Southern¹³. Following transfer, the filters were incubated in a solution of 50% formamide, 5 $\times$ SSC, 50 mM PO₄ (pH 6.5), 500 mg ml⁻¹ sperm DNA and 5 $\times$ Denhardt's solution at 42 °C for 16 h. The probe for hybridization was derived from the plasmid pDR- β -1 which contains DNA sequences complementary to the coding region and 3' non-translated sequences of class II antigen β -chain messenger RNA^{8,10,21}. The pDR- β 1 clone was digested sequentially with *Pst*I and *Pvu*II and fragments *Pst*I-*Pvu*II and *Pvu*II-*Pvu*II covering nucleotides 1-790 of the clone⁸ were isolated by agarose gel electrophoresis followed by electroelution into dialysis tubing. The purified cDNA fragments were pooled and labelled with ³²P-dCTP (NEN) to specific activities of $\sim 10^8$ c.p.m. μ g⁻¹ by nick-translation. The radioactively labelled cDNA fragments were heat-denatured and incubated with the filters for 16 h at 42 °C at a final concentration of 0.5×10^6 c.p.m. ml⁻¹ in 50% formamide, 5 $\times$ SSC, 20 mM PO₄ (pH 6.5), 200 μ g ml⁻¹ salmon sperm DNA, 1 $\times$ Denhardt's solution and 10% dextran sulphate 500 (Pharmacia). The filters were then washed three times in 2 $\times$ SSC, 0.1% SDS at room temperature for 5 min and twice in 0.1 $\times$ SSC, 0.1% SDS at 50 °C for 45 min before they were exposed to Kodak XAR-5 film with an intensifying screen at -70 °C for 7 d.

The β -chain cDNA probe^{8,10} which detects sequences in the human major histocompatibility complex on chromosome 6 (ref. 8) was first used to test whether the hybridization bands were different between HLA-DR identical IDDM and healthy individuals. DNA was isolated from peripheral lymphocytes from 22 IDDM patients, diagnosed before the age of 18 years at the department of paediatrics, University of Linköping, Sweden, being either HLA-DR3/- ($n = 5$), DR4/- ($n = 8$), or

Table 1 Variable and invariable* restriction fragments detected (kb)

	<i>Eco</i> RI	20	13	11*	9.0	6.5	4.0	3.5	2.2
<i>Bam</i> HI	12	10*	8.0*	7.0*	6.2	5.8	5.0*	4.3	3.2
<i>Pst</i> I	18	10	7.2	6.5	6.3	6.0	5.4	5.1	4.9

Table 2 *Pst*I 18 kb and *Bam*HI 3.7 kb restriction endonuclease fragments detected by the HLA-D region β -chain probe

	<i>Pst</i> I 18 kb			<i>P</i> value†	<i>Bam</i> HI 3.7 kb		
	No. of individuals	No. of positive	(%)		No. of positive	(%)	<i>P</i> value†
HLA-DR3*							
IDDM	10	4	(40)	NS	0	(0)	NS
Controls	4	2	(50)		0	(0)	
HLA-DR4*							
IDDM	15	15	(100)	$P < 0.04\ddagger$	0	(0)	$P < 0.04\ddagger$
Controls	9	6	(67)		3	(33)	
HLA-DR3/4							
IDDM	22	21	(96)	$P < 0.02\ddagger$	1	(5)	$P < 0.05\ddagger$
Controls	13	8	(62)		4	(31)	
All individuals							
IDDM	47	40	(85)	$P < 0.025\ddagger$	1	(2)	$P < 0.002\S$
Controls	26	16	(62)		7	(27)	

* Both IDDM and control individuals were selected to carry only HLA-DR3 (3/3 or 3/-) and HLA-DR4 (4/4 or 4/-), respectively.

† Fisher's exact test was used to test differences between IDDM and control individuals. Multiplying the *P* values with the number of fragments observed resulted in the following corrected *P* values (P_c): $\ddagger P_c > 0.05$; $\S P_c < 0.02$. NS, Not significant.

DR3/4 ($n = 9$) positive. Additional IDDM patients ($n = 25$) being either HLA-DR3 (3/3 or 3/-) ($n = 5$), HLA-DR4 (4/4 or 4/-) ($n = 7$) or HLA-DR3/4 ($n = 13$) and healthy controls being HLA-DR3 ($n = 4$), HLA-DR4 ($n = 9$) or HLA-DR3/4 ($n = 13$) were selected from files of frozen lymphocytes of previously DR-typed individuals^{5,11}. HLA-DR antigens 1-5, 7-8 were typed as described¹¹.

Second, we compared the hybridization patterns in DNA isolated from randomly selected IDDM patients ($n = 29$) and healthy controls ($n = 25$)¹². All patients were diagnosed under the age of 30 and were treated with insulin. All patients and healthy controls were caucasians.

The DNA samples ($\sim 5 \mu\text{g}$) were digested with the restriction endonucleases *Eco*RI, *Bam*HI or *Pst*I; the DNA fragments separated by agarose gel electrophoresis and transferred to nitrocellulose filters by the method of Southern¹³. Filters were hybridized with a ³²P-labelled cDNA probe prepared by nick translation from isolated *Pvu*II fragments of the recombinant plasmid pDR- β -1^{8,10} (see legend to Fig. 1 for details).

The pDR- β -1 probe hybridized to 5-9 *Bam*HI, 4-7 *Eco*RI and 4-8 *Pst*I restriction fragments in the different individuals tested (Table 1). *Bam*HI sequences of 10, 8.0, 7.0 and 5.0 kb and *Eco*RI sequences of 11 and 6.5 kb were present in all individuals; the other sequences were highly variable. Family studies demonstrate that these sequences are closely linked to HLA-DR (manuscript in preparation). The complex patterns of bands detected with our β -chain probe contrast with that of the α -chain class II antigen cDNA probes which give only a single hybridization signal^{18,14,15}. However, the number of fragments is less than the multitude of fragments detected with probes for class I antigens^{16,17}. Figure 1 shows the sequences detected in three unrelated HLA-DR3/4 healthy individuals after digestion with *Bam*HI, *Eco*RI or *Pst*I. *Bam*HI sequences of 3.7 and 12 kb, an *Eco*RI sequence of 20 kb and *Pst*I sequences of 18 and 5.1 kb varied between the individuals (Fig. 1).

The frequencies of the 3.7 kb *Bam*HI and 18 kb *Pst*I sequences differed between HLA-DR identical IDDM and healthy individuals (Table 2). The *Pst*I 18 kb sequence was present in increased frequency among HLA-DR4 and DR3/4 positive IDDM patients. Patients with the HLA-DR4 (4/-, 4/4 and 3/4) haplotype differed from controls even if the *P* value was corrected for the number of *Pst*I fragments observed ($P_c < 0.01$). The frequency of the 3.7 kb *Bam*HI sequence was clearly decreased in IDDM patients compared with controls ($P_c < 0.01$). We also noted that six of the eight HLA-DR3 and/or 4 positive individuals having the 3.7 kb sequence lacked the 18 kb *Pst*I fragment.

The above investigations were carried out on highly selected individuals based on previous HLA-DR typing. We also determined the distribution of *Bam*HI, *Eco*RI and *Pst*I restriction fragments in 54 unrelated individuals to obtain an idea of the

population frequency of generated fragments. Healthy controls ($n = 25$) were randomly selected from a blood bank and 29 individuals were randomly selected IDDM patients¹². None of these individuals were available for HLA-typing.

The restriction enzyme polymorphism analysis demonstrated that, compared with the healthy blood donors, the IDDM patients had a decreased frequency of the 2.2 and 9.0 kb *Eco*RI sequences ($P_c < 0.02$), and of the 3.2 and 3.7 kb *Bam*HI sequences ($P_c < 0.001$) (Table 3). The 5.8 kb *Bam*HI ($P_c < 0.05$) and 4.9 and 6.0 kb *Pst*I ($P_c < 0.05$) sequences showed increased frequencies. These differences might be explained by the greater occurrence of HLA-DR3 and/or HLA-DR4 specificities in the IDDM patients⁵. However, the absence of the 3.7 *Bam*HI fragment in IDDM which is present in 40% of healthy controls cannot be simply interpreted. First, the 3.7 kb *Bam*HI fragment was not detected in homozygous HLA-D/DR2 standard typing cells¹⁸. The decreased frequency of the *Bam*HI 3.7 kb fragment is therefore not explained by the negative association between

Table 3 The distribution of restriction fragments in randomly selected IDDM patients and controls

Sequence size (kb)	IDDM <i>n</i> = 29	Controls <i>n</i> = 25	<i>P</i> value
<i>Eco</i> RI			
2.2	2 (10%)	12 (48%)	0.002†
3.5	23 (79%)	16 (64%)	NS
4.0	26 (79%)	20 (80%)	NS
9.0	3 (10%)	12 (48%)	0.002†
13.0	21 (73%)	15 (60%)	NS
20.0	25 (86%)	14 (56%)	0.01*
<i>Bam</i> HI			
3.2	3 (10%)	15 (60%)	0.0001§
3.7	0 (0%)	10 (40%)	0.0001§
5.8	23 (79%)	10 (40%)	0.004†
6.2	3 (10%)	10 (40%)	0.01*
<i>Pst</i> I			
4.0	7 (26%)	3 (12%)	NS
4.9	21 (78%)	9 (36%)	0.003†
5.1	10 (37%)	18 (72%)	0.01*
5.4	4 (15%)	12 (48%)	0.01*
6.0	26 (96%)	16 (64%)	0.004†
6.3	18 (67%)	7 (28%)	0.006*
18.0	23 (85%)	18 (72%)	NS

3.2 versus 3.4 kb, 4.0 versus 4.3 kb and 10.0 versus 12.0 kb *Bam*HI sequences were not resolved; only sequences which varied between individuals are shown. *Pst*I sequences 10, 7.2 and 6.5 have not been scored. Differences between IDDM and controls (Fisher's exact test) correcting for the number of fragments: * $P_c > 0.05$; † $P_c < 0.05$; ‡ $P_c < 0.01$; § $P_c < 0.001$. NS, Not significant.

HLA-D/DR2 and IDDM⁵. Second, a positive hybridization signal for a 3.7 kb *Bam*HI sequence was only observed in HLA-DR5 positive homozygous typing cells¹⁸. Third, HLA-DR5 was present in only 6.1% of IDDM patients and 8.7% of healthy controls⁵. The 3.7 kb *Bam*HI sequence present in 40% of our randomly selected healthy individuals may therefore be associated with an HLA specificity not found in the homozygous typing cells examined¹⁸. This is not surprising since the frequency of blank HLA-DR determinations in the general population amounts to 20–30% (ref. 19). Taken together the results in Tables 2 and 3 show that the 3.7 kb *Bam*HI sequence is detected rarely in IDDM patients but more frequently (30–40%) among healthy controls even if they are HLA-DR4 positive.

The randomly selected IDDM patients and blood donors did not differ in the frequency of the 18 kb *Pst*I fragment. They were not analysed for the 10, 7.2 and 6.5 kb *Pst*I fragments

which showed weak hybridization signals. The difference in the 18 kb *Pst*I frequency shown in Table 2 therefore only applies when comparing HLA-DR identical individuals and may simply be explained by the possibility that HLA-DRY is associated with more than one determinant^{19,20}.

While the molecular characterization of the 3.7 kb *Bam*HI and 18 kb *Pst*I sequences in HLA-DR4 positive individuals must await the isolation of the genes containing them, our results demonstrate a novel approach to the understanding of the susceptibility to not only IDDM but also other disorders previously found to be associated with the HLA complex. Furthermore, analysis of the restriction fragments generated in families with multiple affected IDDM members may help clarify the genetic basis and the mode of inheritance of the disease.

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Autoantibodies to the insulin receptor in juvenile onset insulin-dependent diabetes

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Insulin-dependent diabetes mellitus (IDDM) usually begins in childhood or early adulthood, and its aetiology is thought to involve autoimmune damage to the islet cells that secrete insulin¹. To investigate an additional target of autoimmunity in IDDM we examined sera for antibodies to insulin receptors. Such antibodies were defined by their ability to compete with insulin for binding to insulin receptors and by their capacity to behave like insulin in activating lipogenesis in adipocytes. We now report the occurrence of anti-insulin receptor antibodies of the IgM class in the sera of 10 of 22 IDDM patients obtained before their treatment with exogenous insulin. Furthermore, two of five IDDM patients who were initially negative developed anti-insulin receptor antibodies during treatment with human or pork insulin. These findings suggest that autoimmunity to the insulin receptor may contribute to the pathophysiology of IDDM.

The population of IDDM patients we examined comprised children who were referred consecutively to the Department of Endocrinology of the Sophia Children's Hospital. Sera were obtained from 22 patients before treatment and from 5 of them also after they began to receive insulin. Control sera were obtained from healthy volunteer blood donors at the Blood Bank of the Leiden University Hospital and from the investigators. We surveyed the sera by assaying 0.5–5 µl for insulin-like activity in stimulating lipogenesis in rat adipocytes measured by incorporation of radiolabelled glucose into lipid^{2,3}.

Table 1 shows that the insulin-like activity in a representative serum could be identified as anti-insulin receptor antibody of

the IgM class by fractionating the serum and testing the various fractions for lipogenic activity. For example, 75% of the lipogenic activity found in the whole serum could be eluted from an anti-µ column that specifically binds IgM. The effluent which contained the non-IgM material had relatively little lipogenic activity. Filtration through Sephacryl 300 to separate IgM from IgG confirmed that the lipogenic activity was a property of the fraction containing IgM, while the fraction containing IgG was inactive. Dissociation of possible insulin-insulin antibody complexes by acidification and separation of serum fractions on Sephadex G-100 showed that the lipogenic activity remained intact and resided in the insulin-free, high-molecular weight fraction². The low-molecular weight fraction did not contain sufficient insulin to activate lipogenesis. The lipogenic activity of the positive sera was not inhibited by adding antibodies to insulin, illustrating by another method that the lipogenic activity was not due to insulin itself. In contrast, goat antibodies to human IgM inhibited ~95% of the lipogenic activity in the positive sera. Lipogenic IgM did not bind to immobilized insulin (not shown). These results taken together indicate that the lipogenic activity could be assigned to IgM molecules and not to free insulin or insulin-insulin antibody complexes. The lipogenic IgM was identified as anti-receptor antibody as it specifically competed with radiolabelled insulin for binding to insulin receptors. Table 2 illustrates that increasing amounts of IgM purified on an anti-µ column increasingly inhibited the binding of insulin to the insulin receptors of adipocytes. Control IgM isolated from the serum of an IDDM patient without lipogenic activity had no effect on the binding of insulin to its receptor.

Normal human IgG stimulates lipogenesis in rat adipocytes *in vitro*⁴. Although the IgM anti-receptor antibodies described here also caused lipogenesis, the two lipogenic effects differ with regard to both ligand and receptor. The stimulatory effect of IgG is exerted through the non-variable Fc portion of the molecule^{4,5} and normal IgG does not compete with insulin for binding to the insulin receptor (P. Dandona, personal communication).

Table 3 documents the presence of anti-insulin receptor antibodies in the sera of IDDM patients before and after treatment with exogenous insulin. Ten out of 22 patients had these antibodies in their sera at the time they first presented, before they had been treated with exogenous insulin. We have had the opportunity to examine serial bleedings obtained after

Table 1 Anti-insulin receptor antibodies in serum of IDDM patient are IgM

Treatment of serum	Serum fraction	% Relative lipogenesis of adipocytes
None	Whole	100
Anti- μ column	Effluent (not IgM)	15
	Euate (IgM)	75
Sephacryl 300	Large (IgM)	100
	Small (IgG)	0
Dissociation of complexes, Sephadex G-100	Large (Ig)	90
	Small (insulin)	0
Antibodies to insulin*	Whole	100
Antibodies to IgM†	Whole	5

Sera were fractionated² using columns of goat antibodies to human μ -chain (Dako Immunoglobulins), bound to Sepharose (Pharmacia¹¹), Sephacryl 300 (Pharmacia¹²) or Sephadex G-100 (Pharmacia). Antigen-antibody complexes were dissociated by acidification (0.01 M HCl, pH 2.7) of serum² before gel filtration. Control serum or fractions for each procedure were obtained from healthy donors and from an IDDM patient whose whole serum was negative for anti-receptor antibody activity. Lipogenic activity was computed relative to that found in 1 μ l of the unfractionated serum (100%). Adipocytes were obtained from the epididymal fat pad of male Wistar rats (90–120 g) and the incorporation of D[U-¹⁴C]glucose (4–7 mCi mol⁻¹; NEN) into lipid was measured as described previously^{2,3}. Maximal lipogenesis (100%) was equivalent to that produced by incubation of the adipocytes with insulin (10 ng ml⁻¹) and was 300% of control lipogenesis obtained without added insulin.

* Guinea pig anti-insulin antiserum (Miles-Yeda; titre 10⁻⁵) was added (2 μ l) to the lipogenic assay.

† Goat antiserum to IgM (Miles-Yeda) was added (30 μ l) to the lipogenic assay.

Table 2 Purified IgM receptor antibody competes with insulin for binding to insulin receptors on rat adipocytes

Affinity-purified IgM (μ g)	% Inhibition of insulin binding to adipocytes	
	Test serum	Control serum
5	29	9
10	48	4
20	72	0

Affinity-purified IgM eluted from an anti- μ column (see Table 1) was added in the indicated amounts to rat adipocytes (10⁵ cells) in plastic tissue culture tubes containing 0.35 ml KRB buffer (pH 7.4)–0.3% bovine serum albumin and ¹²⁵I-insulin (35,000 c.p.m.). The tubes were incubated at 25°C for 40 min and the adipocytes were then separated from unbound insulin on a Millipore filter (EGWP, 0.2 μ m), washed with ice-cold buffer and counted for radioactive content¹³. Extent of binding was 1.7 fmol per 10⁵ adipocytes of which 70% was specific (displaced by 1 μ M cold insulin). Per cent inhibition was computed relative to the binding obtained in the absence of added IgM.

treatment of five patients who had been negative at the outset. Two of these patients became positive within 4 months of receiving treatment with exogenous insulin, one having been given human and the other porcine insulin.

The investigation described here was prompted by the observation that mice developed anti-insulin receptor antibodies spontaneously after immunization to insulin². These receptor antibodies were identified as anti-idiotypes to insulin antibodies, suggesting that they might have arisen as components of an idio-type-anti-idio-type network⁶. The anti-idiotypes probably functioned as receptor antibodies by mimicking the conformation of the antigen insulin⁷. We reasoned that humans might possibly develop similar anti-idiotypic insulin receptor antibodies in response to their own insulin antibodies produced by accidental immunization to exogenous insulin used for treatment. However, a large number of the pretreatment sera which we had believed would serve

Table 3 Anti-insulin receptor antibodies in sera of IDDM patients before and during treatment with exogenous insulin

Serum donors	Anti-insulin receptor antibodies
Normal controls	0/20
IDDM patients	
Before treatment	10/22
After treatment	2/5

Anti-receptor antibodies in each serum were identified by two or more of the assays described in Table 1. Sera of 22 patients were obtained before they were treated with injections of insulin. Sera were obtained serially from five patients treated with injections of insulin after being negative for receptor antibodies before treatment. Two patients became positive for anti-receptor antibodies during 4 months of observation.

as negative controls were found to be positive for anti-receptor antibodies (Table 3). Therefore, we must conclude that autoimmunity to insulin receptors, rather than resulting merely from an iatrogenic accident, may be generated during the pathological processes intrinsic to IDDM. Moreover, the prevalence of these antibodies in an unselected group of patients suggests that their presence is neither sporadic nor infrequent (Table 2). We have no evidence to indicate whether or not the anti-insulin receptor antibodies in the IDDM patients are anti-idiotypes to insulin antibodies.

Insulin receptor antibodies have been found in a few dozens of patients with acanthosis nigricans and severe diabetes⁸. It seems, however, that IDDM and the acanthosis nigricans diabetic syndrome are diverse entities with distinct anti-receptor antibodies. Unlike IDDM, the anti-receptor antibodies in the acanthosis nigricans patients are mostly IgG rather than IgM⁸, the disease is extremely rare, the resistance to treatment with insulin is marked and the patients seem to have a primary structural defect of their insulin receptors⁹.

Patients with IDDM, with or without IgM receptor antibodies, do not have the degree of insulin resistance characteristic of the acanthosis nigricans syndrome. The disparate clinical entities associated with these anti-receptor antibodies may be attributed to the diverse biological effects of IgG and IgM receptor antibodies, to differences in the fine specificities of the receptor antibodies, to the intrinsic state of the insulin receptors and/or to the presence or absence of additional pathological processes.

Regardless of the mechanism of insulin receptor antibody generation, their presence indicates that autoimmunity in IDDM is not limited to islet cells¹. The clinical consequences and theoretical implications of these anti-receptor antibodies may be important. What is their role, alone or together with viral infection and islet cell antibodies, in the pathogenesis of IDDM? Do they influence the response to treatment or the development of late complications? Can they be used to identify degrees of risk or immune-response genes? Are they a factor in the subclinical insulin resistance that is a prominent feature of IDDM¹⁰?

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Local regulation of compensatory noradrenergic hyperactivity in the partially denervated hippocampus

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Functional recovery after denervating lesions in the central nervous system (CNS) is particularly prominent if part of the lesioned projection is spared. Several plasticity mechanisms, such as collateral sprouting, hyperactivity of remaining axons and development of receptor supersensitivity, probably contribute to efficient recovery after subtotal lesions¹⁻⁴. Although denervation-induced collateral sprouting⁵⁻⁸ and presynaptic compensatory hyperactivity in spared axons⁹⁻¹³ have been described in various systems, any possible interaction or cooperation between the two mechanisms in restoring synaptic transmission in a partially denervated target has so far not been demonstrated. We have shown previously that partial adrenergic denervation of the hippocampus in adult rats is followed by a slow and protracted reinnervation by collateral sprouting from the spared adrenergic afferents¹⁴. We now report that the partial adrenergic deafferentation is accompanied by a transient increase in turnover of the transmitter in remaining axons which subsides when the denervated region becomes reinnervated, and that the development of this compensatory hyperactivity is confined to the area of maximal denervation. The topographical specificity of the compensatory noradrenergic hyperactivity response, and the interaction between this hyperactivity and the collateral reinnervation process, strongly suggest that the changes in transmitter turnover in spared afferents after denervating lesions can be regulated by local mechanisms operating within the denervated target area.

Young adult female Sprague-Dawley rats (Anticimex AB) were subjected to bilateral or unilateral lesions of the fimbria-fornix and supracallosal pathways, as described previously¹⁴. The lesion was made by aspiration with a fine pipette under visual control in the dissection microscope so as to ensure complete transection of the hippocampal fimbria, the dorsal fornix and the overlying cingulate cortex. In addition, the superior cervical ganglia were removed bilaterally to avoid interference by sympathetic axons growing into the denervated hippocampus^{15,16}. Non-lesioned, sympathectomized rats were used as controls. At 3 weeks and 8 months after surgery (bilateral lesions) and 1 week, 3 weeks and 3 months after surgery (unilateral lesions) the hippocampus was taken for measurement of catecholamine synthesis rates according to the dopa accumulation method of Carlsson *et al.*¹⁷. In this method, accumulation of dopa is measured 30 min after injection of the dopa decarboxylase inhibitor, NSD 1015 (100 mg per kg, subcutaneous (s.c.); Sandev). In a preliminary experiment, we determined that hippocampal noradrenaline (NA) levels did not significantly change after 100 mg per kg NSD 1015 if animals were killed within 30 min after the injection. At 40 min, there was usually a fall in the level of noradrenaline. Noradrenaline and dopa were assayed radioenzymatically¹⁸⁻²⁰ in dorsal, middle and ventral hippocampus on both sides (see Fig. 1), and in the left and right cerebellar hemispheres.

As illustrated schematically in Fig. 1, the adrenergic afferents from the locus coeruleus reach the hippocampal formation along two entirely separate routes: a dorsal route, via the fimbria-fornix and cingulum, and a ventral route, via the piriform lobe. The dorsal route provides most of the adrenergic innervation (~85-90%) of the dorsal hippocampus (slice I in Fig. 1), while the ventral route is the main source of innervation

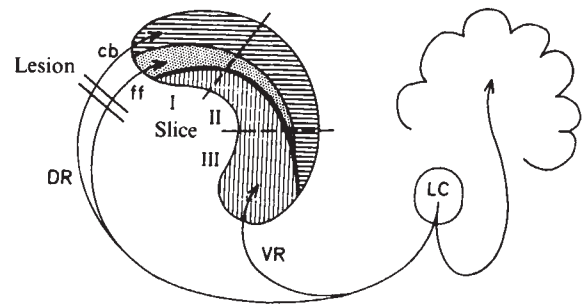


Fig. 1 Schematic illustration of the noradrenergic afferents from the locus coeruleus (LC) reaching the hippocampal formation along two separate routes: a dorsal route (DR) via the fimbria-fornix (ff) and cingulate bundle (cb) and the ventral route (VR) via the piriform lobe (see text for further explanation of the experimental paradigm).

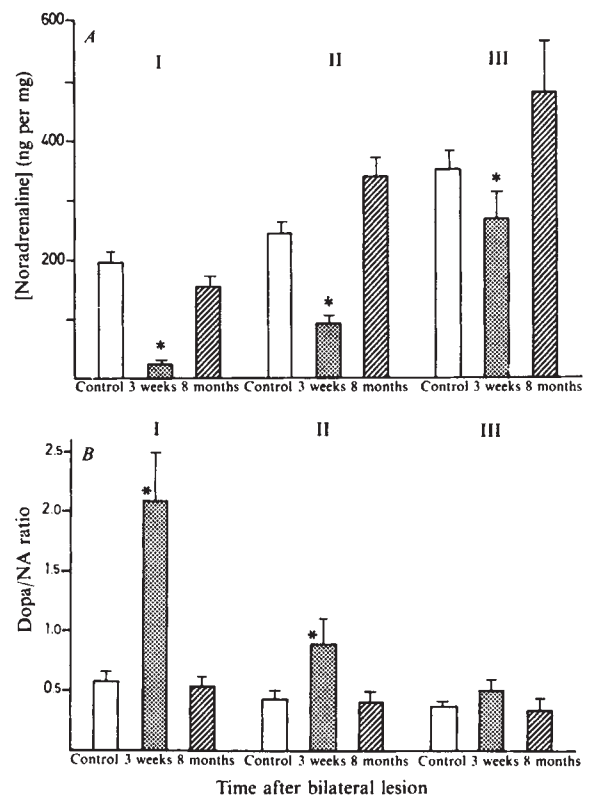


Fig. 2 **A**, Noradrenaline concentration in the hippocampal formation at 3 weeks and 8 months after bilateral fimbria-fornix and cingulate bundle lesions. Slice I corresponds to the septal third, slice II to the posterior third, and slice III to the ventral third of the hippocampus. **B**, The ratio of dopa to noradrenaline obtained from three divisions of the hippocampus 30 min after NSD 1015 injection (100 mg per kg) at 3 weeks and 8 months after lesion of the dorsal adrenergic route. *, $P < 0.01$ compared with control values.

(~80%) to the ventral hippocampus (slice III in Fig. 1). Bilateral lesion of the dorsal pathways thus caused, by 3 weeks after operation, a 90% reduction in NA in the dorsal hippocampus (slice I), 55% reduction in the middle hippocampus (slice II) and 20% reduction in the ventral part (slice III) (stippled columns in Fig. 2A). By 8 months the NA concentrations had recovered to near-normal levels in all three segments (cross-hatched columns in Fig. 2A). The NA levels had undergone a six-fold increase in slice I and a three-fold increase in slice II ($P < 0.001$ in both cases). This is consistent with our previous

findings, in unilaterally lesioned rats¹⁴, that the initially denervated segments are slowly reinnervated by collateral sprouting from the spared afferents.

The rate of dopa accumulation in the NSD 1015-treated animals revealed marked changes in the catecholamine synthesis rate during the denervation-reinnervation process (Fig. 2B). Thus, in the partially denervated hippocampus there was, by 3 weeks after operation, a significant increase in the dopa/NA ratio. Interestingly, the increase was greatest (3.5-fold) in the dorsal hippocampus (slice I) where reduction in NA was most pronounced, and it was absent from the ventral segment (slice III) which was only marginally denervated by the fimbria-fornix lesion (stippled columns in Fig. 2B). Moreover, by 8 months after operation, when the NA levels were normalized, the dopa/NA ratio had returned to normal in all segments (cross-hatched columns in Fig. 2B).

The time course and regional selectivity of the lesion-induced changes in catecholamine synthesis rate were further analysed in animals having unilateral fimbria-fornix lesions. The changes in hippocampus ipsilateral to the lesion were compared with those in the corresponding segments of the contralateral hippocampus (Fig. 3). The cerebellar hemispheres were analysed in parallel as an example of a locus coeruleus projection area remote from the lesion (Fig. 4). This area was chosen partly because the hippocampus and cerebellum are known to be innervated in part by collaterals from the same locus coeruleus neurones²¹, and partly because remote changes in the NA innervation of the hippocampus and cerebellum have been reported after lesions of the locus coeruleus projections to the cerebellum and hippocampus, respectively^{22,23}.

Consistent with the observations in the bilaterally lesioned animals, the unilateral fimbria-fornix lesion caused an increase in the dopa/NA ratio in the dorsal and middle portions (slice I and II in Fig. 3) but not in the ventral portion (slice III) of the denervated ipsilateral hippocampus (Fig. 3). This effect was not present at 7 days after the lesion, when the anterograde degeneration of the lesioned NA axons was still incomplete (an average of 30% reduction of NA in slice I). The hyperactivity was fully developed by 3 weeks, that is, at a time when the axonal degeneration was completed (83% reduction of NA slice I), and it still remained by 3 months, at which time compensatory collateral sprouting has previously been reported to be underway¹⁴ (70% reduction of NA in slice I). No significant changes in either NA concentration or dopa/NA ratios were recorded in the contralateral hippocampus (open columns B, in Fig. 3) or cerebellum (Fig. 4) at any time point.

These results provide evidence for an adaptive mechanism in the adrenergic innervation of the hippocampus whereby an increased turnover of transmitter in spared afferents can partly restore adrenergic neurotransmission in the denervated target. This compensatory hyperactivity response has several interesting features. First, the effect was developed at 3 weeks but not at 1 week after lesion. As the disappearance of the locus coeruleus terminals in cortical areas (including hippocampus) after axotomy takes about 2 weeks^{24,25}, this slow time-course suggests that the increased NA turnover is a response to the mechanisms involved with adrenergic denervation rather than to cessation of impulse flow in the lesioned axons. Second, the magnitude of the response seemed to be related to the degree of adrenergic denervation and to be confined to those areas which were substantially denervated by the lesion. Third, the NA turnover parameter was long-lasting and normalized only at a time when the reinnervation of the initially denervated target had been completed.

Compensatory adrenergic hyperactivity has previously been demonstrated in the sympathetic nervous system after extensive 6-hydroxydopamine (6-OHDA)-induced sympathectomy²⁶. Similarly, increased catecholamine turnover has been demonstrated in the central dopamine and NA projection systems after extensive 6-OHDA lesions⁹⁻¹³. The effect induced by peripheral sympathectomy is probably mediated by a trans-synaptic induction of tyrosine hydroxylase through a reflex

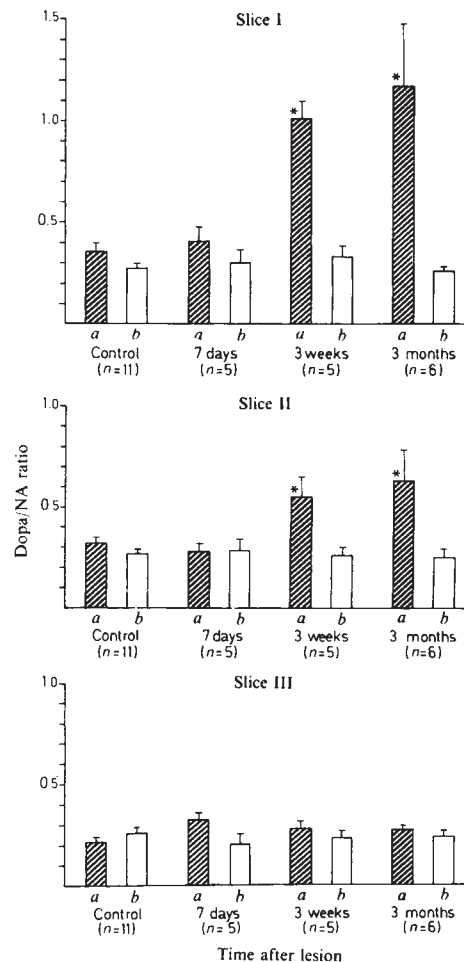


Fig. 3 Dopa to noradrenaline ratios obtained from three divisions of the hippocampus ipsilateral (a) and contralateral (b) to unilateral lesion of the dorsal adrenergic route after NSD 1015 injection 7 days, 3 weeks and 3 months following the lesion. *, $P < 0.01$ compared to control values.

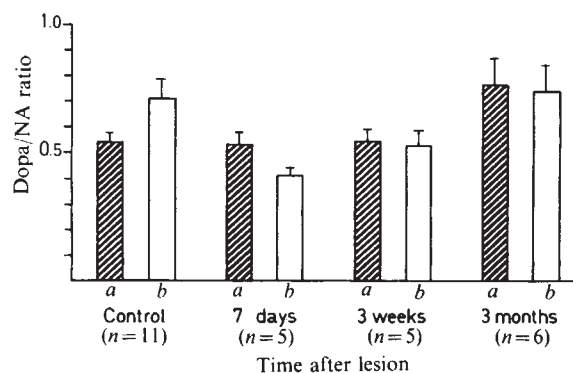


Fig. 4 Dopa to noradrenaline ratios obtained from the cerebellum ipsilateral (a) and contralateral (b) to a unilateral fimbria-fornix plus cingulate bundle lesion after NSD 1015 injection 7 days, 3 weeks and 3 months following the lesion.

activation of the cholinergic nerves innervating the spared adrenal medullary cells^{27,28}. A similar feedback regulatory mechanism has been proposed also after CNS lesions¹⁰. It remains a possibility that a cell body-mediated mechanism could account for the local response described here. This seems unlikely, however, as it would require that the observed changes took place in a subpopulation of neurones which send their collateral branches via both the dorsal and ventral routes, but

whose collaterals, which pass along the ventral route, traverse the ventral hippocampus without sending out collaterals, to terminate exclusively in the dorsal hippocampus. Such an explanation seems particularly unlikely in view of our current understanding of the wide collateralization of the locus coeruleus neurones, and the fact that our lesion completely deafferented the hippocampus of its adrenergic innervation via the dorsal routes. Available anatomical data²⁹ indicate, in fact, that the dorsal and ventral hippocampus are largely innervated by the same population of locus coeruleus neurones. Therefore, the regionally confined adrenergic activation observed with more restricted lesions in the present study indicates the importance of local, rather than cell body-mediated, regulatory mechanisms in the maintenance of adrenergic neurotransmission in a partially denervated target. Several types of mechanisms are conceivable in such local transmitter turnover regulation: (1) pre-synaptic autoreceptors located on the adrenergic terminals may normally mediate an inhibition of NA synthesis via NA released by neighbouring terminals^{30,31}. Hyperactivity following partial denervation would thus result from a removal of this 'lateral inhibition' mechanism³². (2) Similar to that which has been proposed for the dopaminergic terminals in the neostriatum³³, local interneurons may mediate a feedback inhibition of NA turnover in the adrenergic terminals. The denervation-induced hyperactivity would then be due to a release from this inhibitory feedback. (3) Finally, the local regulation could reflect a trophic interaction with the target tissue. If so, neuronotrophic factors, postulated to be released in the hippocampus on deafferentation³⁴, could act not only as sprouting inducing agents but also as regulators of enzymes involved in the transmitter biosynthesis. Whatever mechanism underlies this compensatory hyperactivity, our results suggest that, in the present system at least, this adaptive mechanism interacts with the more slowly developing reinnervation process to restore adrenergic function after denervating brain lesions.

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Human β -nerve growth factor gene sequence highly homologous to that of mouse

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Nerve growth factor (NGF) is thought to have a profound effect on the development and maintenance of sympathetic and embryonic sensory neurones (see refs 1–3 for review). NGF activity isolated from the male mouse submaxillary gland (MSG) consists of three types of subunits, α , β and γ , which specifically interact to form a 7S, ~130,000-molecular weight (M_r) complex. The 7S complex contains two identical 118-amino acid β -chains, which are solely responsible for the nerve growth-stimulating activity of NGF. While NGF is found in almost all vertebrates⁴, most research has focused on murine NGF, as the mouse male submaxillary gland contains higher levels of this polypeptide than other tissues⁴. Even so, β -NGF comprises only ~0.1% of the protein in this small gland, which has made the study of this polypeptide difficult. The amino acid sequence of the mouse NGF β -chain has been determined⁵ and some information has been obtained regarding the size of a mouse precursor molecule⁶, pro- β -NGF, but little was known about the structure and relatedness of β -NGF from other vertebrates. Here we describe the isolation of mouse β -NGF complementary DNA (cDNA) and present its nucleotide sequence, which predicts a prepro- β -NGF molecule of M_r 27,000 (27K) and a pro- β -NGF molecule of M_r 25K. We have used the mouse β -NGF cDNA clone to isolate the human β -NGF gene, the coding regions of which are highly homologous to the mouse prepro- β -NGF nucleotide and amino acid sequences.

The cDNA cloning approach took advantage of the known 118-amino acid sequence of the mouse β -NGF subunit, using synthetic oligonucleotide primers; the difference in NGF levels between male and female MSGs⁷ was used as an additional means of identification. Three small portions of the β -NGF amino acid sequence were chosen, and oligonucleotide pools complementary to all possible sequences coding for them were synthesized⁸. The primer pool representing sequences closest to the carboxy-terminus of the protein (pool 1; see Fig. 1 legend) was used to specifically prime reverse transcription of male MSG poly(A)-containing RNA, in order to first enrich for β -NGF-specific nucleotide sequences. cDNA molecules greater than 200 bp long were cloned into pBR322; 10,000 clones were screened using the 5'-³²P-labelled β -NGF primer pool originally used in the cDNA priming as hybridization probe; ~8% of the clones gave a positive signal in high stringency hybridization conditions.

Positive clones were rescreened using radiolabelled primer pools 2 and 3, derived from sequences upstream from oligonucleotide pool 1, as hybridization probes (see Fig. 1 legend). In addition, ³²P-cDNA primed with pool 1 from male or female poly(A)⁺ MSG RNA was used as a probe on duplicate filters. Nine male-specific clones which hybridized with oligonucleotide pools 2 and 3 were identified. The clone containing the longest DNA insert (pm β N-9G1; 716 bp) was sequenced in its entirety. The amino acid sequence deduced from the nucleotide sequence contained the expected β -NGF sequence in one translational frame in addition to an amino-terminal pro-sequence (Fig. 3).

Northern blot analysis⁹ using radiolabelled, cloned mouse β -NGF sequences as hybridization probe established the size of the mouse β -NGF mRNA as 1,300 nucleotides long (data not shown). Primer extension analyses determined that the various overlapping clones shown in Fig. 1 contain all but 30

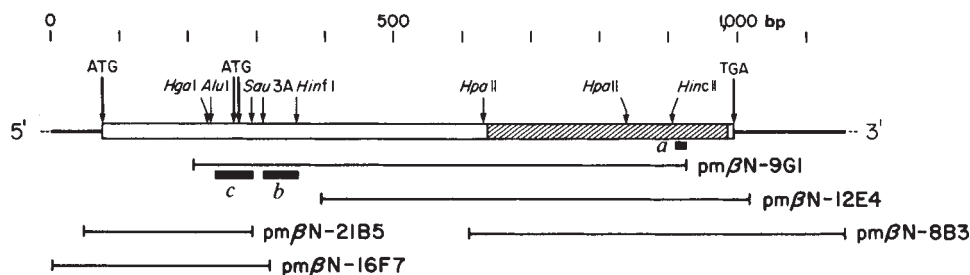


Fig. 1 Construction and identification of bacterial clones containing mouse β -NGF cDNA sequences. Bars *a*, *b* and *c* designate the positions of DNA fragments used to prime cDNA synthesis (see below). The upper line and boxed region represent the entire region of mouse β -NGF cDNA covered by our clones. The bp scale begins arbitrarily at zero, which depicts the

beginning of our nucleotide sequence. The boxed region encloses the open reading frame; the cross-hatched area encodes mature β -NGF. The three potential sites of translation initiation (ATG) are indicated, as is the TGA termination codon. Only those restriction sites which were used to generate specific hybridization probes or primers, and were mentioned in the text, are shown.

Methods: Primer pool 1 (3'-CTGCTTCTTGTCCG-5', complementary to sequences between amino acids 93 and 97) was used to specifically prime the synthesis of cDNA on male MSG poly(A)⁺ RNA; 220 pmol (1 μ g) of each pool of 8 primers (440 pmol in total, 2 μ g) were annealed with 40 μ g poly(A)⁺ RNA in 50 μ l of 100 mM KCl by incubating for 4 min each at 90, 68, 42 and 37 °C. ³²P-labelled double-stranded (ds) cDNA was synthesized and treated with S₁ nuclease in standard conditions²⁸. After phenol-chloroform extraction and ethanol-precipitation the cDNA was fractionated by electrophoresis on a 5% polyacrylamide gel. cDNA >200 bp was electroeluted and cloned into pBR322 as described²⁸; 10,000 colonies were screened by filter hybridization²⁹: 1 μ g of primer pool 1 was phosphorylated with 200 μ Ci [γ -³²P]ATP (5,000 Ci mmol⁻¹; Amersham) and polynucleotide kinase (P-L Biochemicals). Filters containing the 10,000 clones were hybridized with $\sim 1 \times 10^8$ c.p.m. of the ³²P-labelled probe at room temperature²⁹. About 8% of the colonies (830) gave positive hybridization signals. Three sets of identical filters were prepared from the positive colonies identified in the first round of screening. Filters were then hybridized using radiolabelled pools 1 (above), 2 and 3 (3'-TTCTGTACCTTGA-5' and 3'-ATGAAGAAAGCTCTG-5', complementary to sequences between amino acids 74 and 77 and 52 and 56, respectively). Nine colonies hybridized with all three oligonucleotide pools. Plasmid DNA was isolated and the clone having the largest insert was determined by restriction enzyme analysis. The 716-bp insert of clone pm β N-9G1 was completely sequenced by the Maxam-Gilbert method²¹. For the isolation of clones containing complete 5' sequences, we used two restriction fragments isolated from pm β N-9G1. The ds 58-bp *AluI*-*Sau3A* fragment (*c*) and the 50-bp *Sau3A*-*HinfI* fragment (*b*) were used separately to prime cDNA synthesis: ~ 0.2 μ g of each fragment (~ 6 pmol) were phosphorylated with [γ -³²P]ATP (Amersham) and polynucleotide kinase. Annealing and ds cDNA synthesis procedures were basically as before. Priming was, however, more specific, resulting in a detectable radioactive band in a polyacrylamide gel for each priming reaction. The bands were electroeluted from gel slices, and after phenol-chloroform extraction and ethanol-precipitation the cDNA was cloned into pBR322. A 123-bp *AluI*-*HinfI* fragment of pm β N-9G1 was used as a radioactive hybridization probe. pm β N-21B5 and pm β N-16F7 had the largest DNA inserts and were completely sequenced by the Maxam-Gilbert method. pm β N-21B5 contained the entire priming strand sequence (*c*) and a 250-bp cDNA insert, whereas pm β N-16F7 contained only the last 6 bases of the priming strand sequence (*b*) and a 320-bp cDNA insert. pm β N-8B3 and pm β N-12E4 were isolated from 4,400 clones by hybridization with a 216-bp mouse β -NGF *HpaII* fragment, obtained by oligo(dT)-primed cDNA synthesis on a size fraction of male MSG poly(A)⁺ RNA (~ 800 –1,500 bases). RNA fractionation on a urea-agarose gel⁹ and cDNA cloning were as described elsewhere²⁸. Neither of these cDNA clones contains the entire 3'-untranslated sequence of mouse β -NGF cDNA.

nucleotides from the 5'-end of the mRNA, and lack at the most 150 nucleotides from the 3'-end.

The 1,164-nucleotide cDNA sequence (Fig. 3) contains a long (327 amino acids) open reading frame which includes the known amino acid sequence of mouse β -NGF^{5,10} (amino acids 1–118). The predicted β -NGF sequence differs at one position from that determined by amino acid sequencing procedures: we predict an aspartic acid at residue 30, rather than asparagine^{5,10}. The β -NGF sequence is flanked at its amino- and carboxy-terminal ends by polypeptide extensions which presumably represent portions of a prepro- β -NGF precursor. The boundary sequences include basic amino acids (Lys-Arg, Arg-Arg) at both ends, which are found at precursor processing sites in other systems^{11–14}. Processing at the amino-terminal boundary excludes both basic residues from the mature polypeptide, while cleavage at the carboxy-terminal end seems to occur between the two arginine residues. The resulting mature β -NGF chain possesses a carboxy-terminal arginine residue, thought to be essential for the stability of the 7S NGF complex¹⁵. The entire carboxy-terminal propeptide is only 2 amino acids long, which is unprecedented in other prohormone systems.

Determination of the exact size of the amino-terminal region is difficult, as no amino-terminal polypeptide sequencing information for *in vitro*-translated prepro- β -NGF or pro- β -NGF is yet available. Evidence has been presented for the existence of a 22,000-*M*, biosynthetic mouse pro- β -NGF precursor⁶, but the precursor predicted by the nucleotide sequence is longer than that previously detected.

Three methionine residues are possible candidates for the protein synthesis initiation codon (amino acids –187, –121 and –119), but several factors strongly implicate amino acid –121 of our sequence as the actual initiation codon used. The methionine codon closest to the 5'-end of mammalian mRNAs is usually used to initiate protein synthesis, however, there are many exceptions to this general scheme¹⁶. As β -NGF is a

secreted protein, the initiation codon is probably followed by a signal sequence for co-translational transfer of this polypeptide into the lumen of the endoplasmic reticulum¹⁷. The stretch of amino acids following the methionine at position –187 (closest to the 5'-end) contains a high percentage of polar and charged amino acids and bears no resemblance to any previously described signal sequence¹⁷. On the other hand, the amino acids between methionine residues –121 or –119 and residue –104 represent an excellent candidate signal sequence. The 16–18 amino acids following residue –121 or –119 include a stretch of six completely hydrophobic amino acids (Ala-Phe-Leu-Ile-Gly-Val). Cleavage by signal peptidase could occur between the small amino acid –104 (alanine) and the glutamic acid residue at –103. Signal peptidase cleaves after an identical alanine-glutamic acid sequence to leave an analogous amino-terminal glutamic acid residue in the case of pre- α -lactalbumin¹⁸. Because methionine –121 follows Kozak's rule in that it possesses a purine nucleotide three residues upstream¹⁶, we favour amino acid –121 over methionine –119 as the actual initiator methionine. The use of methionine –121 for translation initiation of mouse prepro- β -NGF would result in a 27K preprohormone and a 25K pro- β -NGF if signal peptide processing occurs at residue –104. The availability of the sequence reported here and the corresponding cDNA and chromosomal gene clones should allow further elucidation of the specificity and order of intracellular processing events for the β -NGF precursor protein.

A human gene library¹⁹ was screened using a 665-base pair (bp) mouse β -NGF cloned cDNA fragment (pm β N-9G1 *HgaI*-*HincII*; Fig. 1) as radioactive hybridization probe; 27 hybridization-positive recombinant phage were plaque-purified and partially characterized by *EcoRI* digestion. Six different types of restriction pattern were identified; each pattern shared restriction fragments and thus appeared to overlap the same genomic region. Clone λ h β N8 was characterized further by

Fig. 2 Physical map and partial nucleotide sequence of the human β -NGF gene. **A**, A human gene library¹⁹ was screened using a radio-labelled³⁰ 470-bp *Rsa*I fragment of pm β N-9G1. λ h β N8 was analysed by restriction enzyme cleavage with *Eco*RI (RI), *Hind*III (H3) and *Bam*HI (B); cleavage sites are indicated by vertical arrows. Of the 17.3-kb human DNA insert, 15 kb were sequenced. Zigzag marks represent the Charon 4A phage arms. The boxes demarcate presumptive mature mRNA regions; the cross-hatched area of the larger box encodes mature β -NGF, which would be transcribed from left to right (arrow). The size scale at the top is shown in kilobase pairs (kbp), from the 5' end of the phage insert. **B**, The nucleotide sequence was determined from pBR322-subcloned *Eco*RI and *Hind*III fragments²¹. Numbers at the right refer to nucleotide numbers. The regions homologous to the mouse β -NGF cDNA clone are translated, and the region corresponding to mature human β -NGF is boxed. Potential initiation methionines are underlined with dashed lines, at positions analogous to the mouse sequence (see Fig. 3). The position of a transcription initiation sequence, TATAAA (double underlined), is also shown. Three possible AATAAA polyadenylation signals are underlined. The nucleotide sequence of 6.6-kb of the intervening sequence is not shown due to space considerations, but was completely determined, and is available on request.



multiple restriction digests, Southern blotting and nucleotide sequencing; Fig. 2A shows the resulting physical map. A 3,900-bp nucleotide sequence derived from subcloned, overlapping *Eco*RI and *Hind*III fragments is shown in Fig. 2B.

Regions within phage λ h β N8 which are homologous to our mouse β -NGF cDNA sequence were identified by Southern hybridization experiments²⁰, followed by nucleotide sequence analysis²¹ and translation of the sequence into amino acid sequence information. Two regions of extensive homology were identified that were 124 and 738 bp long, respectively, separated by a 6.6-kilobase (kb) intervening sequence. The 738-bp region encodes a protein that is highly homologous (90%) to mature mouse β -NGF (see below, and Fig. 3) and apparently

represents the thus far undetermined human β -NGF protein sequence. Like mouse β -NGF, the putative human β -NGF sequence also contains two amino acid codons downstream from the presumed carboxy-terminus of the mature polypeptide, followed by a signal for translational termination (TGA). Farther downstream there are multiple AATAAA signals for mRNA polyadenylation^{22,23}, but the first one occurs 978 nucleotides beyond the translation termination site. Unless the 3'-untranslated region of the sequence is interrupted by an intervening sequence, the length of the predicted transcribed region would be substantially longer than the mouse mRNA.

The human pre- and pro-peptide sequences upstream from the mature β -NGF region are highly homologous to the mouse

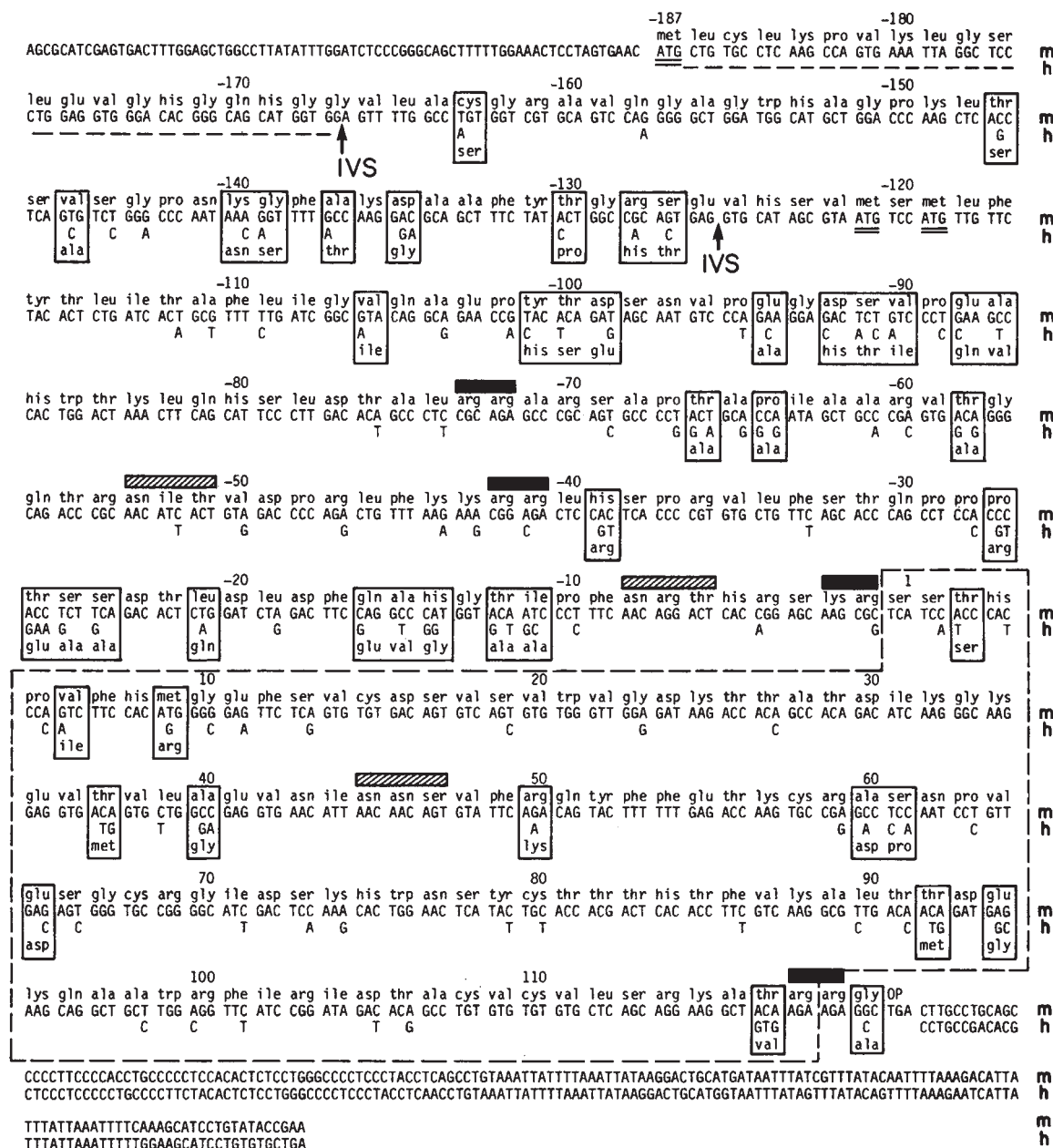


Fig. 3 Nucleotide sequence of cloned mouse prepro-β-NGF cDNA and comparison with human sequences. The mouse cDNA sequence was derived from overlapping clones (Fig. 1) by the procedure of Maxam and Gilbert²¹. Only the coding strand is shown; the longest open reading frame starting with a methionine residue is also given. A termination codon is present upstream at the twelfth nucleotide of the top line. Potential initiation codons are double-underlined; as described in the text, we favour methionine -121. Amino acid positions are numbered, beginning with the first amino-terminal residue of the mature β-NGFs, the sequences of which are boxed in by a broken line. The mouse coding strand nucleotide and amino acid sequences (m) are shown in their entirety; only differences are shown below for the corresponding human (h) sequences. The positions at which a nucleotide difference causes a change in the coded amino acid are boxed; cross-hatched bars indicate potential N-linked glycosylation sites; solid bars potential protease cleavage sites. IVS refers to the position of intervening sequences in the human gene. OP, opal termination codon, TGA. Sequences upstream from the intervening sequence (IVS) at position -166 are only known for the mouse cDNA; dashes under amino acids -167 to -186 denote missing human sequences.

cDNA and identical in length. The 6.6-kb intervening sequence occurs 12 nucleotides upstream from the methionine residue we have designated as the translation initiation site (-121). If our initiation codon assignment is correct, the second homologous region of 124 nucleotides represents part of the 5'-untranslated region, and is flanked by potential splice sequences²⁴. Interestingly, we were unable to identify a region on phage λhβN8 or any of our other phage isolates which was equally homologous to the 5'-end of the mouse β-NGF cDNA. Either the rest of the 5'-end of the human β-NGF gene including regulatory sequences is separated from the 124-bp homologous mRNA sequence by a >6 kb intervening sequence and is therefore not present on λhβN8, or human and mouse sequences have diverged more extensively in this region. A Goldberg-

Hogness sequence (TATAAA) is present at nucleotide position 462, with distantly homologous sequences downstream (600-660), thus the 5'-end sequences may be more distantly related. However, use of the 5' mouse cDNA probe on human genomic Southern blots at low hybridization stringency detected a homologous sequence elsewhere in the human genome (data not shown), supporting the first interpretation. Isolation of the human prepro-β-NGF will be necessary to clarify this point. In addition, high-stringency Southern hybridization experiments using human and mouse chromosomal DNA indicated the presence of a single β-NGF gene per haploid genome (data not shown).

Comparison of mouse β-NGF nucleotide and amino acid sequences with corresponding human sequences reveals high

levels of homology. Figure 3 compares the 861 bp that comprise the small and large exons of the human gene with the mouse cDNA sequence. The nucleotide sequences are highly homologous overall (86%). Although we have tentatively assigned the amino-terminus for mouse prepro- β -NGF to Met -121, we included the amino acid sequences of the open reading frame which precede amino acid -121 in both mouse cDNA and human gene for comparison. Most of the sequence upstream from position -121 (40 amino acids) corresponds to the small exon in the human gene, and may actually represent 5'-untranslated sequences. The degree of homology in this region (positions -126 to -166) is significantly lower than that overall (70%). Highly conserved regions within the putative prepro- β -NGF amino acid sequence are the pre-peptide (-121 to -104; 94.5%) and the sequences of mature β -NGF (90%). Within the amino- and carboxy-terminal pro-sequences, regions of more extensive divergence separate several areas of high homology between mouse and human. Interestingly, the regions which surround two Arg-Arg dipeptides (positions -41/-42 and -72/-73) and two potential N-linked glycosylation sites at amino acids -8 and -53 are absolutely conserved, which may reflect the functional importance of these sequences. A third Arg-Arg dipeptide is present at the carboxy-terminal end of the pro- β -NGF sequence and appears to be a protease cleavage site in both human and mouse sequences for the removal of a carboxy-terminal dipeptide. Within the mature β -NGF sequence the cysteine residues, important for the formation of proper tertiary structure¹⁰, are conserved, as are 90% of the other residues; amino acid differences are mostly conservative with respect to the chemical nature of the residues. Further evidence for structural homology between mouse and human β -NGFs has been obtained by expression of the human β -NGF sequence in *Escherichia coli* and immunological detection of a protein of the correct size using an anti-mouse β -NGF antiserum (A.U. *et al.*, in preparation).

Because of low (10–23%) levels of homology in the amino acid sequences of the B chains of NGF and the insulin gene family members (insulin, relaxin and insulin-like growth factors), it has been postulated that β -NGF is a member of the insulin gene family³. We therefore compared the nucleotide sequences of the β -NGF and insulin genes^{25,26}; the level of homology is greater than random in the B-chain region but does not further strengthen the hypothesis that these genes are related. Both genes contain an intervening sequence just upstream from the coding sequences in the 5'-untranslated region, but this is not a particularly unusual gene feature²⁷. Isolation and characterization of other genes in the insulin gene family will be necessary to shed light on this interesting hypothesis³.

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The Hardy-Zuckerman 2-FeSV, a new feline retrovirus with oncogene homology to Abelson-MuLV

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There is substantial evidence that oncogenes (*v-onc*) of acute transforming retroviruses have been acquired by transduction of cellular genes (*c-onc*) with retroviruses^{1,2}. Feline leukaemia virus (FeLV)-associated feline fibrosarcomas have proven to be extremely useful for the isolation of acute transforming retroviruses of a mammalian species. Three different *v-onc* genes have been identified in five acute transforming feline retroviruses. The Susan McDonough feline sarcoma virus (SM-FeSV)³ contains the oncogene *fms* (ref. 4). The Snyder-Theilen (ST)⁵ and Gardner-Arnstein (GA)⁶ FeSVs contain the oncogene *fes* (ref. 4), which is homologous to the oncogene *fps* of the avian sarcoma viruses FSV, RRCII, PRCIV and 16L (refs 7, 8). The *v-onc* sequences of the Parodi-Irgens (PI) FeSV have recently been found to be homologous with the *v-sis* sequences of the simian sarcoma virus^{9,10}. We report here the isolation of another acute transforming feline retrovirus from a naturally occurring feline fibrosarcoma, designated the Hardy-Zuckerman 2 feline sarcoma virus (HZ2-FeSV) and demonstrate that the HZ2-FeSV and Abelson murine leukaemia virus (A-MuLV) have homologous oncogenes.

The HZ2-FeSV was isolated from tumour tissue of an FeLV-infected 4-yr-old male Siamese cat (SKI 3590) who had 20–30 large fibrosarcomas in the subcutaneous tissues and muscle of the right foreleg, face, thorax and abdomen (Fig. 1)¹¹. The serotype of FeLV present in the serum and tumour tissue of this cat was determined to be FeLV-A and -B by Oswald Jarrett (Glasgow University). One of three 6-week-old kittens inoculated subcutaneously in the dorsolumbar area with 0.75 g equiv. of a 0.45 μ m filtrate of a homogenate from one of the primary 3590 tumour nodules developed multiple fibrosarcomas of the heart and kidneys 16 weeks after inoculation.

Infection of mink CCL64 cells with filtered homogenate of the primary tumour produced discrete foci of transformed cells. These foci consisted of rounded cells that grew in clusters and detached from the underlying cuboidal CCL64 mink cells. Transformed virus-nonproducer (NP) cells were obtained from which transforming virus could be rescued with amphotropic 292-MuLV¹² or with a mixture of FeLV A, B and C derived from feline FL74 cells¹³. These results indicated that the HZ2-FeSV is a replication-defective acute transforming retrovirus.

The integrated HZ2-FeSV genome in cellular DNA of the mink HZ2-FeSV-infected (NP) cells was analysed as follows. High molecular weight DNA from HZ2-FeSV-infected mink

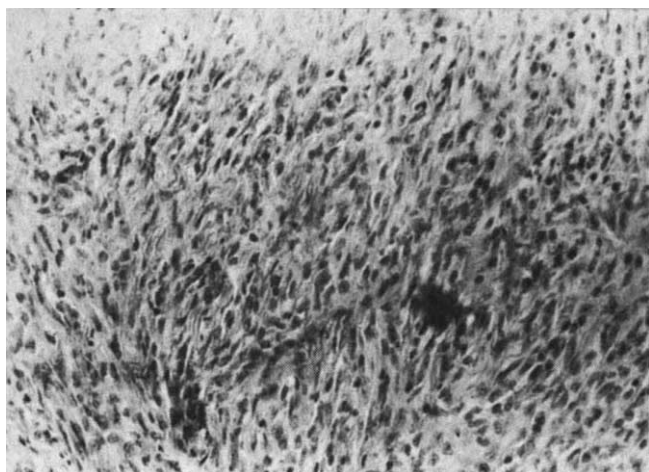


Fig. 1 Histological appearance of one of the many subcutaneous fibrosarcomas present in cat SKI 3590 from which HZ2-FeSV was isolated. The tumour is composed of a compact sheet of fusiform cells with relatively few mitotic figures present, indicating a relatively slowly enlarging tumour. $\times 160$.

cells and from uninfected mink cells was digested with the restriction endonucleases *Eco*RI, *Bam*HI and *Hind*III and analysed by blot hybridization. As shown in Fig. 2a, the following FeLV-related restriction fragments were detected: *Eco*RI, 12.5 kilobases (kb); *Bam*HI, 13.5 kb (faint) and 5 kb; *Hind*III, 9.5 and 3.7 kb. This restriction fragment pattern suggests that these cells contain a single FeSV provirus which contains sites for the enzymes *Bam*HI and *Hind*III but not for *Eco*RI.

The *v-onc* sequences of the HZ2-FeSV were characterized by determining whether they were related to any of the known retroviral oncogenes. We first considered *v-fes*, the most common oncogene found in feline and avian acute transforming retroviruses⁷. A duplicate Southern blot containing DNA from HZ2-FeSV-infected mink cells and from uninfected mink cells, as described above, was hybridized with a *v-fes* probe to deter-

mine if the fragments containing HZ2-FeSV sequences identified by the FeLV *rep* probe (Fig. 2a) also contain *v-fes* sequences. As shown in Fig. 2c, hybridization with the *fes* probe revealed only mink *c-fes* sequences that were present in the DNA samples of both infected and uninfected mink cells. Because no *fes* sequences were found to be associated with FeLV sequences, the HZ2-FeSV does not contain a *fes* oncogene. Similar experiments showed that the HZ2-FeSV *v-onc* sequences were not related to *v-fms* and *v-sis* (data not shown).

We then determined the relationship of the HZ2-FeSV *v-onc* to the *abl* sequences of A-MuLV^{14,15}, the only acute transforming retrovirus that contains *v-abl*. In the P160 strain of A-MuLV the *v-abl* sequences constitute a 4.2-kb segment in the 6.3-kb viral genome¹⁶. A duplicate Southern blot containing DNA from HZ2-infected mink cells and from uninfected mink cells as discussed above was hybridized with a *v-abl* probe (pAB3 sub 3, *Bgl*II-*Hind*III fragment 2.6–4.9 kb of the A-MuLV genome)¹⁵. In relaxed hybridization conditions the *v-abl* probe detected *c-abl* sequences which were present in the DNA of both infected and uninfected mink cells and DNA restriction fragments which were detected only in the DNA of the HZ2-FeSV-infected cells, namely a 12.5-kb *Eco*RI fragment, a 5-kb *Bam*HI fragment and a 3.7-kb *Hind*III fragment (Fig. 2b). Comparison of the size of the restriction fragments detected by the *v-abl* probe reveals that all of the *v-abl* fragments also contained FeLV sequences, indicating that the FeLV and the *v-abl* sequences are linked in the HZ2-FeSV genome.

The specificity of the hybridization reaction between the A-MuLV *v-abl* probe and the HZ2-FeSV sequences was investigated by hybridizing Southern blots containing mink HZ2-FeSV DNA, and normal mink, cat and mouse DNA, with the *v-abl* probe, washing at 60°C in 2×SSC (low stringency) or at 65°C in 0.2×SSC (high stringency), and comparing the hybridization signals (Fig. 2d). On stringent washing, the HZ2-FeSV signal was slightly reduced and the mink *c-abl* and cat *c-abl* signals were reduced to the same intensity as the HZ2-FeSV signal. The mouse *c-abl*, on the other hand, was not changed significantly. This therefore shows that the hybrids of

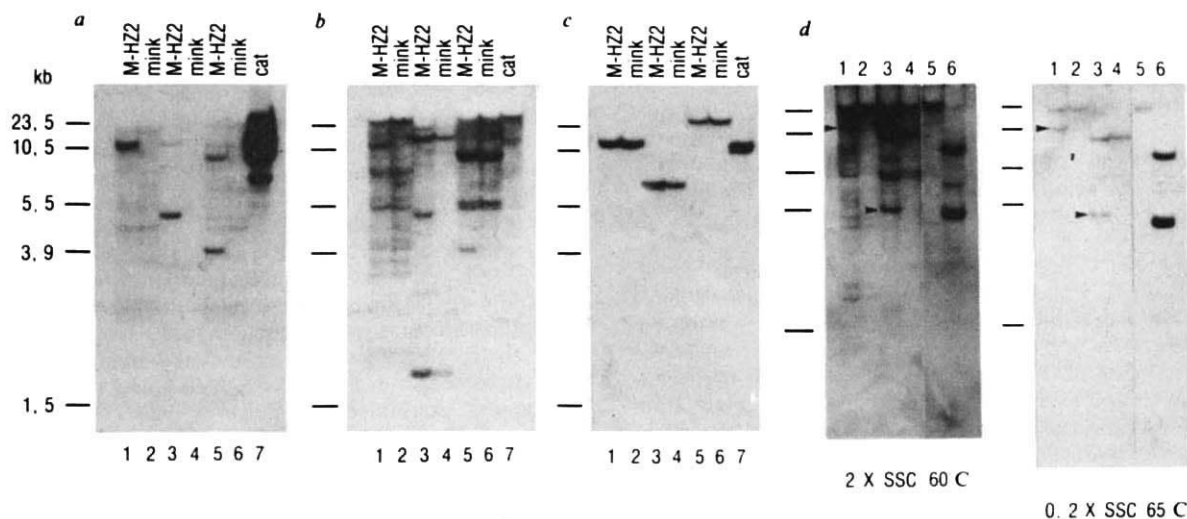


Fig. 2 a–c, Identification of *abl* sequences in the integrated HZ2-FeSV provirus by Southern blot analysis. d, Specificity of *v-abl* hybridization to HZ2-FeSV sequences.

Methods: In a–c 10 µg each of high molecular weight DNA from HZ2-FeSV-infected mink cells and from uninfected mink cells was digested with the restriction endonucleases *Eco*RI, *Hind*III and *Bam*HI. NIH mouse DNA was digested with *Hind*III and cat FLF-3 DNA was digested with *Eco*RI. The DNAs were fractionated in a 1% agarose gel and transferred to nitrocellulose paper. The filters were hybridized with the following ³²P-labelled hybridization probes (2–5 × 10⁸ c.p.m. per µg): a, pB-FeLV-B; b, pAB3 sub 3 (8), c, pB-*v-fes* essentially as described elsewhere¹⁰. The DNAs analysed were as follows: mink HZ2-FeSV DNA digested with *Eco*RI (lanes 1), *Hind*III (lanes 3), *Bam*HI (lanes 5), mink DNA digested with *Eco*RI (lanes 2), *Hind*III (lanes 4), *Bam*HI (lanes 6), and cat FLF-3 DNA digested with *Eco*RI (lanes 7). Migration of *Bam*HI-digested Charon 4a DNA fragments is indicated at the left. For d, a Southern blot, as described above, containing DNA from HZ2-FeSV-infected and uninfected mink cells cleaved with different restriction enzymes was hybridized with ³²P-labelled *v-abl* probe. After washing the blot in 2×SSC, 0.1% SDS at 60°C and in 0.2×SSC, 0.1% SDS at 65°C, the hybridization signals were visualized by autoradiography. The DNAs analysed were as follows: mink HZ2-FeSV DNA digested with *Eco*RI (lanes 1), *Hind*III (lanes 3), mink DNA digested with *Eco*RI (lanes 2), *Hind*III (lanes 4), cat FLF-3 DNA digested with *Eco*RI (lanes 5) and NIH mouse DNA digested with *Hind*III (lanes 6).

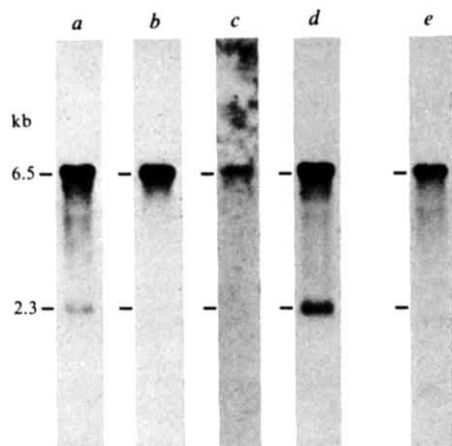


Fig. 3 Blot analysis of RNAs specified by the HZ2-FeSV genome in mink cells.

Methods: 5 µg of cytoplasmic poly(A)⁺ RNA were denatured with glyoxal and dimethyl sulfoxide, fractionated in a 1% agarose gel and transferred to diazophenyl thioether paper^{10,17}. The blot was hybridized with the following ³²P-labelled hybridization probes: *a*, pB-FeLV-B (FeLV rep); *b*, 5'-*gag* (1.13–2.1 kb of the FeLV-B restriction map¹⁸); *c*, 3'-*gag-pol* (2.1–4.6 kb of the FeLV-B genome); *d*, *env* (5.4–7.2 kb of the FeLV-B genome); *e*, *abl* (pAB3 sub3 *v-abl*-specific probe)¹⁵. Size markers included 7.8-kb FeLV-B DNA, 4.3-kb ST-FeSV DNA, 28S and 18S mouse ribosomal RNA.

A-MuLV *v-abl* with HZ2-FeSV and similarly with cat *c-abl* and mink *c-abl* are rather stable and implies a high degree of relatedness between a fraction of these sequences. Note the similar degree of hybridization of HZ2-FeSV and cat *c-abl* after stringent washing. This would be consistent with a feline origin for the *v-abl* sequences in the HZ2-FeSV.

Transcription of the HZ2-FeSV genome in the infected mink cells was analysed by RNA blot hybridizations¹⁷. Figure 3 shows hybridizations of ³²P-labelled DNA fragments corresponding to various regions of the FeLV-B genome¹⁸ and of the *v-abl* probe to blots containing cytoplasmic poly(A)⁺ RNA. Two RNA species, of 6.5 kb and 2.3 kb, were detected with the FeLV rep probe. The same two RNAs were also detected with an *env*-specific probe (5.4–7.2 kb of the FeLV-B genome). The *v-abl* probe, a 5' *gag* and a 3'-*gag-pol* probe (1.1–2.1 kb and 2.1–4.6 kb of the FeLV-B genome, respectively) only react with the 6.5 kb RNA. Hybridization of the 3'-*gag-pol* probe with the 6.5-kb RNA, however, was much weaker than the FeLV-B hybridization control on the same blot (not shown), indicating a minor homology of ~0.2 kb. We presume the 6.5-kb RNA to be the genome of the HZ2-FeSV. It contains *v-abl* and FeLV *gag* and *env* sequences, but lacks most of the *pol* gene. The 2.3-kb RNA, on the other hand, only contains *env* sequences, suggesting that it is a subgenomic mRNA. Consequently, the *v-abl* gene product is specified by the 6.5-kb RNA and not by the 2.3-kb RNA.

The translation products of the HZ2-FeSV genome were investigated by immunoprecipitation analysis of extracts of metabolically labelled infected mink cells using monospecific antisera to FeLV structural proteins. As shown in Fig. 4, a polypeptide with a molecular weight of 98,000 (P98) was the only molecule detected by antiserum directed against FeLV p27. The same protein was also detected by antisera specific for FeLV p15 and p12 but not by antisera directed against FeLV gp70 (data not shown). The translation products of many acute transforming retroviruses have *in vitro*-associated protein kinase activities. The HZ2-FeSV P98 was also assayed for this activity by incubating *Staphylococcus aureus* Cowan I-bound immune complexes containing the polypeptide with [γ -³²P]ATP and Mn²⁺ as previously described¹⁹. Analysis of phosphorylated reaction products by SDS-polyacrylamide gel electrophoresis (PAGE) indicated that phosphorylation of P98 and the heavy chain of IgG had occurred (Fig. 4).

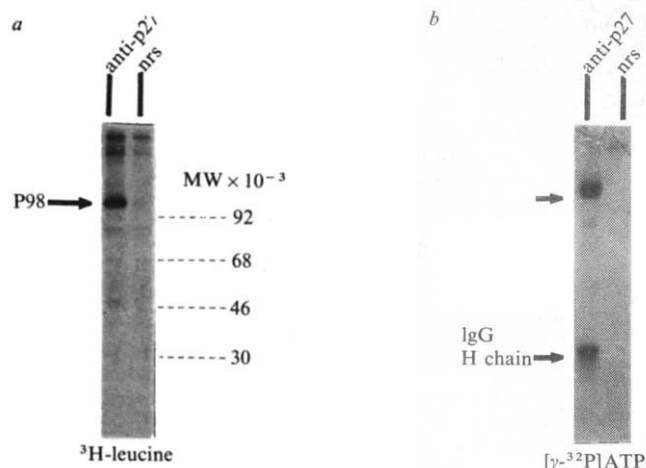


Fig. 4 Characterization of HZ2-FeSV translation product. *a*, SDS-PAGE of ³H-leucine-labelled proteins from HZ2-FeSV-infected mink CCL64 cells immunoprecipitated with anti-FeLV p27 serum (anti-p27) or normal rabbit serum (nrs). *b*, SDS-PAGE of ³²P-labelled proteins in *S. aureus*-bound immune complexes of HZ2-FeSV-P98 after incubation of the complexes in a protein kinase assay system.

Methods: Cells from subconfluent cultures of HZ2-FeSV-infected mink cells, which had been incubated for 18 h at 37 °C in leucine-free Eagle's minimal essential medium containing 5% dialysed fetal calf serum and 50 µCi ml⁻¹ L-[4-³H]leucine (60 Ci mmol⁻¹, NEN) were collected by scraping, washed twice with phosphate-buffered saline (PBS) and resuspended in a 20-fold volume of a lysis buffer comprising 10 mM NaH₂PO₄-Na₂HPO₄, pH 7.5, 100 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 10% glycerol, 1 mM EDTA, 10% aprotinin and 5 mM phenylmethylsulphonyl fluoride. After vigorous vortexing, the extracts were clarified by centrifugation at 100,000g for 30 min. Aliquots of cell extracts (400 µl, 600 µg protein) were incubated with 5 µl of anti-p27 serum or nrs at 37 °C for 60 min and at 4 °C for 60 min. Immune complexes were collected by adsorption to *S. aureus* Cowan I (Pansorbin; Calbiochem) added as 50 µl of a 10% (v/v) suspension in 50 mM Tris HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 0.5% Triton X-100. After incubation for 30 min at 4 °C the immunoprecipitates were collected by centrifugation (5,000g, 5 min) and washed five times with cell lysis buffer. In these conditions all of the available polypeptide is immunoprecipitated¹⁹. For analysis of [γ -³²P]ATP phosphotransferase activity in *S. aureus*-bound immune complexes of the HZ2-FeSV protein, immunoprecipitations from extracts of unlabelled cells were performed as above. Immunoprecipitates were washed five times with lysis buffer, then twice with 20 mM Tris-HCl pH 7.2. They were resuspended in 50 µl of reaction mixture containing 20 mM Tris-HCl, pH 7.2, 100 µM AMP, 5 mM MnCl₂ and 0.4 µM [γ -³²P]ATP (2,000–3,000 Ci mmol⁻¹, NEN). Incubations were then performed at 30 °C for 10 min. Reactions were terminated by adding 1 ml of ice-cold cell lysis buffer containing 200 µM ATP and 10 mM EDTA and immunoprecipitates were collected by centrifugation. For SDS-PAGE analysis of labelled proteins in immunoprecipitates, the pellets were resuspended in 100 µl of SDS-gel sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 2% β -mercaptoethanol, 10% glycerol) and heated at 95 °C for 3 min. *S. aureus* was collected by centrifugation and the supernatants were analysed by SDS-PAGE in step gradient slab gels of 7.5–12.5% acrylamide¹⁹. ¹⁴C-labelled molecular weight (MW) marker proteins were run in the same gel. These included phosphorylase A (92,000), bovine serum albumin (68,000), ovalbumin (46,000) and carbonic anhydrase (30,000) (all obtained from NEN). The gels were fixed and dried and radiolabelled proteins were visualized by autoradiography using Kodak X-omat film with Cronex (Dupont) intensifying screens at -70 °C.

The studies reported here demonstrate that the HZ2-FeSV and A-MuLV were generated by transduction of the same or a closely related cellular gene from different species with FeLV and Moloney murine leukaemia virus (Mo-MuLV) respectively. As the HZ2-FeSV is derived from a pet cat, it is reasonable to assume that the *v-abl* sequences in this virus are of feline origin.

Our analysis of the genetic composition of the HZ2-FeSV provirus transcriptional products revealed FeLV 5'gag and env as well as v-abl sequences. We assume the arrangement of these sequences in the HZ2-FeSV genome to be 5'-gag-abl-env-3' (in more recent experiments we have confirmed this gene order by restriction mapping of molecularly cloned HZ2-FeSV DNA; P.B. and P.B., unpublished). This leads us to predict that the 98,000-molecular weight protein found in HZ2-FeSV-infected mink cells which contains determinants of the FeLV gag proteins p15 and p30 and exhibits a protein kinase activity, is a gag-abl fusion protein and, in analogy with A-MuLV, we presume it to be the transforming protein of this virus²⁰⁻²². Thus, in the HZ2-FeSV and A-MuLV, the v-abl sequences are contained in similar genetic contexts and are expressed in analogous fashion.

To date, at least 16 different retroviral oncogenes are known which can be distinguished by their nucleic acid sequences. Viruses isolated independently from the same species are frequently found to contain related oncogenes. Only twice previously has homology been reported between retroviral oncogenes from different species^{7,23}. The present results and the recent finding of homology between the simian sarcoma virus v-sis sequences and those of another new FeSV isolate (PI-FeSV)¹⁰ provide further evidence for the transduction of homologous cellular genes from different species by retroviruses. The fact that four different v-onc genes have been found in the six strains of FeSV, all isolated from naturally occurring FeLV-induced feline fibrosarcomas, indicates that there is no particular specificity for the transduction of c-onc genes by FeLV. The finding that three of these four v-onc genes are homologous to v-onc genes of viruses isolated from other species is of significance and predicts that the number of cellular oncogenes which can be transduced by retroviruses is limited.

A-MuLV was isolated from a lymphosarcoma of a prednisolone-treated BALB/c mouse infected with Mo-MuLV. A-MuLV induces tumours of the haematopoietic system, predominantly of the B-lymphocyte lineage, and transforms fibroblasts in culture²⁴⁻²⁷. Like A-MuLV, HZ2-FeSV transforms fibroblasts in culture, but, unlike it, the HZ2-FeSV was isolated from a fibrosarcoma and in a preliminary experiment was shown to induce neoplasms of the same cell type. It therefore seems that HZ2-FeSV is able to transform nonhaematopoietic cells *in vivo*, which suggests that viruses containing abl effect a wider range of cell lineages than previously assumed²⁴⁻²⁷. It will therefore be important to investigate the *in vivo* oncogenic spectrum and *in vitro* transformation specificities of the HZ2-FeSV.

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Goose lysozyme structure: an evolutionary link between hen and bacteriophage lysozymes?

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During evolution, the amino acid sequence of a protein is much more variable and changes more rapidly than its tertiary structure. Given sufficient time, the amino acid sequences of proteins derived from a common precursor may alter to the point that they are no longer demonstrably homologous. The ability to make meaningful comparisons between such distantly related proteins must therefore come primarily from structural homology, and only secondarily (if at all) from sequence homology¹. On the other hand, structural homology in the absence of sequence homology might be attributed to convergent rather than divergent evolution. (A common fold might be dictated by functional or folding requirements.) We have previously argued, on the basis of structural and functional similarities, that the lysozymes of hen egg-white and bacteriophage T4 have a common evolutionary precursor, even though their amino acid sequences have no detectable similarity^{2,3}. Here we report the structure of the lysozyme from Embden goose, a representative of a third class of lysozymes⁴⁻¹⁰ that has no sequence homology^{11,12} (or perhaps very weak homology¹³) with either the hen egg-white or the phage enzyme. The structure of goose egg-white lysozyme has striking similarities to the lysozymes from hen egg-white and bacteriophage T4. However, some parts of goose lysozyme resemble hen lysozyme while other parts correspond only to the phage enzyme. The nature of the structural correspondence strongly suggests that all three lysozymes evolved from a common precursor.

The structure determination of goose lysozyme¹⁴ by isomorphous replacement^{1,15} was based on four heavy-atom replacements supplemented by an additional data set collected^{16,17} for one of the derivatives at lower substitution. Electron density maps calculated at 2.8 Å and 3.2 Å resolution showed several helices and allowed extended segments of the polypeptide backbone to be followed, but also contained many ambiguous regions. In order to trace the complete backbone of the molecule we alternated model building^{18,19} with cycles of crystallographic refinement. Details will be given elsewhere. The model of the structure used for the structure comparisons described here was refined to a crystallographic residual of 0.28 at 2.8 Å resolution. Subsequent refinement has reduced the residual to 0.25 at 2.1 Å resolution.

The polypeptide backbone of goose egg-white lysozyme (GEWL) was compared with those of hen egg-white

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Table 1 Lysozyme structural homologies

	GEWL and HEWL	GEWL and T4L	T4L and HEWL
No. of equivalent residues (N_e)	90	91	74
100 N_e/N_1	70%	55%	57%
100 N_e/N_2	49%	49%	45%
R.m.s. discrepancy	3.2 Å	3.2 Å	3.8 Å
Minimum base change per codon for equivalent residues	1.41	1.44	1.46

The table summarizes the comparisons of the three lysozymes by the method of Rossmann and Argos^{23,24}. The procedure uses a complicated algorithm that can result in slightly different sets of equivalent residues, depending on the starting parameters. For this reason, little significance should be attached to small differences in the number of equivalent residues. N_1 and N_2 are, respectively, the number of amino acids in the smaller and larger protein being compared. HEWL has 129 residues, T4L 164 residues and GEWL 185 residues.

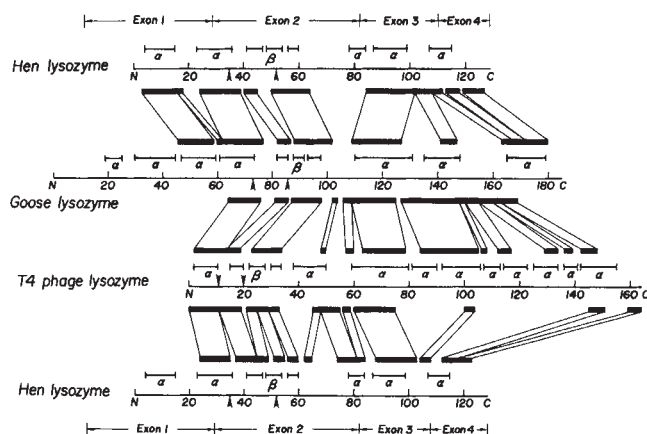


Fig. 1 Structural correspondence between goose, hen and phage lysozymes. The connected solid bars indicate parts of the polypeptide backbones that structurally correspond when the lysozymes are compared in pairs by the method of Rossmann and Argos^{23,24}. The locations of the α -helices and β -strands are shown as are the exons of hen egg-white lysozyme. The arrowheads show the locations of the residues that are presumed to be involved in catalysis, viz Glu 35 and Asp 52 of HEWL, Glu 11 and Asp 20 of T4L, and Glu 73 and Asp 86 of GEWL.

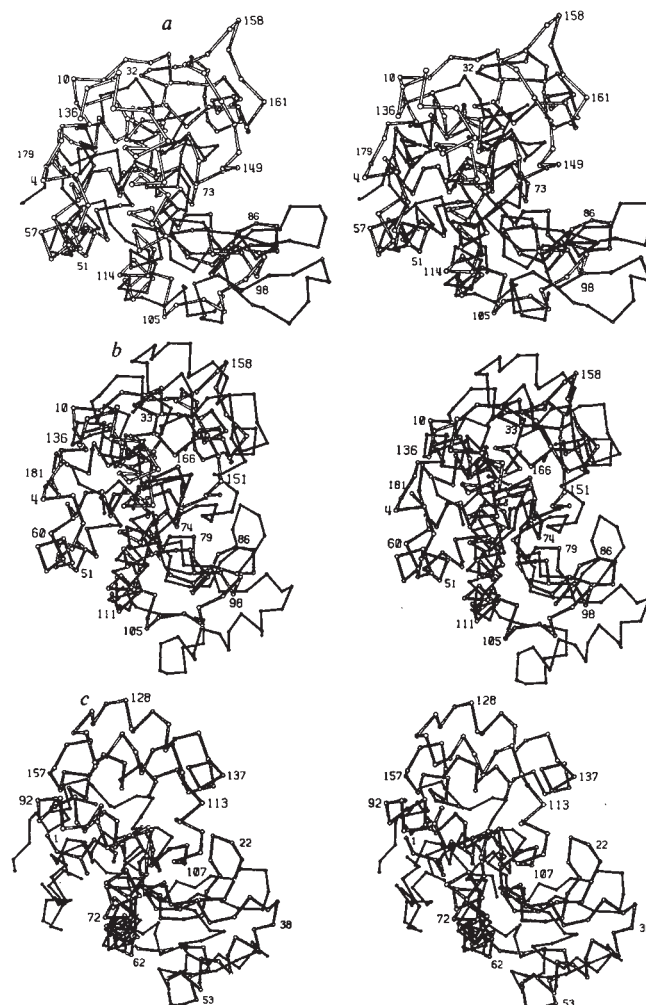


Fig. 2 Stereo drawings showing the superposition of different lysozyme structures. Essentially the same view is used throughout. *a*, GEWL (open connections) and HEWL (solid connections). Numbering for GEWL. *b*, GEWL (open connections) and T4L. Numbering for GEWL. *c*, T4L (open connections) and HEWL (solid connections). Numbering for T4L. (Following Rossmann and Argos²³.)

lysozyme^{20,21} (HEWL) and T4 phage lysozyme (ref. 22, and L.H.W., T. Gray & B.W.M., unpublished results) (T4L) by the methods of Rossmann and Argos^{23,24} and Remington and Matthews^{25,26}. Application of these methods to HEWL and T4L have been described previously^{2,3,23,25}.

The three-dimensional structure of GEWL has striking similarities to both HEWL and T4L (Table 1, Figs 1, 2). Apart from the amino-terminal region, residues 1–46, essentially every residue of GEWL has a counterpart either in HEWL or T4L or both (Fig. 1). There are 90 spatially equivalent α -carbons in GEWL and HEWL, and 91 in GEWL and T4L. For the residues that structurally correspond, the minimum base change per codon is, in each case, close to the random-sequence value of 1.5, confirming the overall lack of amino acid sequence homology between any pair of the proteins.

There are two amino acids in HEWL that are thought to be of prime importance in catalysis, namely Glu 35 and Asp 52^{27,28}. The respective counterparts in T4L are Glu 11 and Asp 20^{29,30}. In the superposition of HEWL on GEWL (Figs 1, 2*a*) the α -carbon of Glu 35 (HEWL) is 0.8 Å from the α -carbon of Glu 73 (GEWL). Also, for T4L aligned with GEWL (Figs 1, 2*b*) the α -carbons of Glu 11 (T4L) and Glu 73 (GEWL) are 1.7 Å apart. Obviously, Glu 73 of GEWL is a counterpart to Glu 35 in HEWL and Glu 11 in T4L. Similarly, Asp 86 of GEWL corresponds to Asp 52 of HEWL and Asp 20 of T4L, although here the agreement is not so precise (see also ref. 31).

According to the Rossmann–Argos procedure, Asp 86 of GEWL is spatially equivalent to Lys 19 rather than Asp 20 of T4L. In the case of HEWL, Asp 86 (GEWL) is topologically equivalent to Asn 44 (Fig. 1) rather than Asp 52, but its α -carbon is only 5.7 Å from that of the latter residue. All three lysozymes have their catalytic aspartates within their common three β -strand unit. For GEWL and T4L, the aspartate is in the first β -strand, whereas Asp 52 of HEWL is in the middle strand. Glu 86 and Asp 73 of GEWL are located on opposite sides of the presumptive active-site cleft. Their carboxyls are about 8 Å apart in the present mode (7–8 Å in HEWL and T4L). Supporting evidence for the location of the active site comes from (*hk*0) and difference Fourier projections (data not shown) indicating that *N*-acetylglucosamine binds to the crystalline enzyme in this region.

When comparing two proteins that have similarities in folding but dissimilar amino acid sequences, there is always a potential ambiguity. The similar conformation might reflect a common evolutionary precursor or, on the other hand, might be a conformation dictated by some functional or folding requirement. This ambiguity occurs quite often (ref. 2 cites a number of instances), and has led to a great deal of controversy concerning divergent versus convergent evolution. Divergent evolution is typified by the well-known families of proteins such as the globins³², the serine proteases³³ and the cytochromes *c* (ref. 34). At the other extreme, subtilisin and α -chymotrypsin³⁵, and

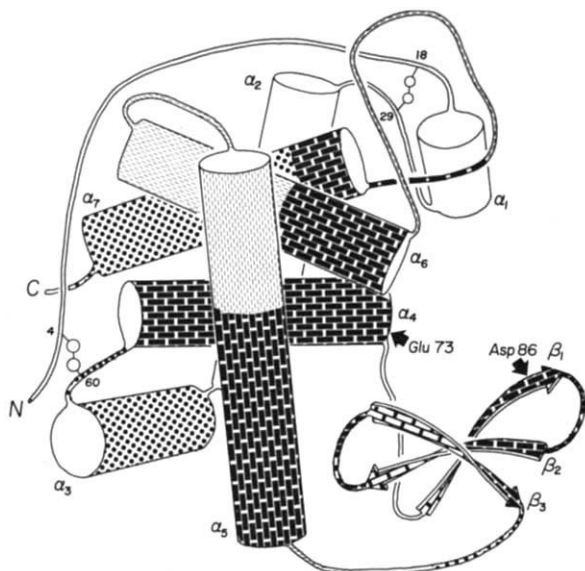


Fig. 3 Simplified drawing of goose egg-white lysozyme showing those parts of the structure that are common to hen and to phage lysozymes. Parts that are common to all three lysozymes are shown as bricks, parts common to GEWL and HEWL are shown dotted, parts common to GEWL and T4L are shown dashed, and parts that occur only in GEWL are shown as open.

thermolysin and carboxypeptidase A³⁶ are normally accepted as examples of convergent evolution. However, in cases such as HEWL and T4L, where the amino acid sequences are not homologous, it is difficult, if not impossible, to choose between divergence and convergence. Now, with three classes of lysozyme structure available, we are in a stronger position. There are parts of four α -helices, together with the three-strand β -sheet, that occur in all three lysozymes, and presumably include the catalytically essential elements of the respective structures. These common residues constitute the core of the molecule and form both sides of the active-site cleft (Fig. 3). All other residues can be regarded as catalytically 'non-essential'. Included among such 'non-essential' residues are 128–141 of GEWL, residues that correspond closely to 85–98 of T4L (average discrepancy 3.0 Å) yet have no structural counterpart in HEWL. Conversely, and perhaps more striking, residues 48–60 of GEWL coincide with the amino-terminal helix, residues 4–16, of HEWL (average discrepancy 2.8 Å), whereas T4L lacks this helix entirely (Figs 1 and 2). It seems unlikely that each of the similarities between the catalytically 'non-essential' parts of the respective lysozymes occurred independently. Rather, these similarities suggest that all three lysozymes diverged from a common precursor, consistent with the accepted principle that secondary structure changes more slowly during evolution than does amino acid sequence^{1,32–34}.

The essence of the structural relation between the three lysozymes is shown in Fig. 4. The triangles, circles and squares are not intended to represent distinct structural domains. Rather, the triangle, for example, illustrates the parts of lysozyme structure that correspond in HEWL and GEWL, but are absent from T4L. This is predominantly the α -helix at the amino-terminus of HEWL, but also includes a segment at the carboxy-terminus (Fig. 1). The three lysozyme types might have arisen by a number of possible evolutionary pathways. One could imagine that a hen-type lysozyme might evolve to (or from) a goose-type lysozyme by appropriate insertions and deletions. The same is true for the phage-type and goose-type enzymes. However, direct evolution from a phage-type to a hen-type lysozyme (or vice versa) seems very unlikely, since it would be necessary to delete some goose-like features from the starting protein and introduce other goose-like features into the product. One possibility is that the three lysozymes diverged from a common precursor that was most akin to a goose-like molecule. It is also possible that the common precursor was



Fig. 4 Formalized representation of the relation between the structures of hen-type, goose-type and phage-type lysozymes together with possible transitions that could have occurred between precursors of these lysozymes during the course of evolution. The symbols are not meant to represent distinct structural domains. Rather, the rectangles represent those structural elements that are common to all three lysozymes, the triangles represent features unique to GEWL and HEWL, the circles represent parts of the lysozyme structures that occur only in GEWL and T4L and the star represents the polypeptide segment that occurs only in GEWL (see Fig. 3).

essentially hen-like, and gave rise to a goose-type descendent that in turn led to the present phage enzyme. The lineage phage-type to goose-type is also possible, but pathways such as phage-type to hen-type to goose-type lysozyme seem very unlikely.

The previous structural comparison of HEWL and T4L² was taken as evidence in support of the view that the four exons of the gene of hen lysozyme³⁷ (Fig. 1) correspond to functional³⁷, and, to some extent, structural units of HEWL^{38–40}. As will be discussed in more detail elsewhere, the present comparisons (Fig. 1) do not strongly suggest that exon units are conserved in the three structures.

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Single-copy DNA sequences specific for the human Y chromosome

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Detailed studies of the role of the mammalian Y chromosome in primary sex determination are limited by the lack of available specific markers and by the fragmentary knowledge of its molecular organization^{1–5}. Y-derived unique DNA sequences could provide powerful analytical tools to probe directly the structure of the Y chromosome and provide a means of searching for specific expressed sequences. We report here the construction of a partial cosmid library of the human Y chromosome. From independent clones we have isolated 30 unrelated DNA probes that are free of highly repetitive sequences, and have examined their reaction pattern on male and female genomic blots. Of the 30 probes tested, six were specific for the Y chromosome. In addition, four probes gave a male–female differential hybridization pattern and the remaining 20, although Y-derived, reacted similarly with both male and female DNA.

We decided to construct a partial cosmid library of the Y chromosome using a somatic cell hybrid containing only the human Y chromosome on a mouse background. A cosmid vector was chosen as isolation of large fragments facilitates chromosome 'walking' and subsequent expression of sequences in eukaryotic cells. In addition, the initial partial restriction used to generate such libraries minimizes the loss of important sequences. Although more direct methods such as chromosome sorting⁶ are faster, the small amount of material generated precludes the use of cosmids. Assuming that the euchromatic portion of Y represents approximately 0.5% of the diploid genome, that is, 3×10^7 base pairs (bp), it is possible to obtain 95% representation with about 2,000 clones of 35–40 kilobases (kb). It should be possible to obtain this number of clones.

The somatic cell hybrid used was that described by Marcus *et al.*⁷. The line was obtained by fusion of a normal male lymphocyte with the mouse cell line, RAG, and contains a modal number of four human Y chromosomes per cell. No other human material can be detected cytogenetically, and extensive screening for numerous human enzymes (including the X-linked steroid sulphatase) was negative (I. Craig and S. Povey, personal communication).

High-molecular weight DNA was extracted from the hybrid, partially restricted with *Mbo*I, and 35–50-kb fragments cloned in *Escherichia coli* using the cosmid vector pJB8 (ref. 8). To differentiate those clones containing human DNA inserts (presumed to be Y-derived) from those containing murine material, the colonies were screened at low density with human ³²P-labelled repetitive DNA⁹. Conditions were arranged so that only repetitive sequences were detected. The DNA used was a *Cot*₁₀₀ fraction of total sheared genomic DNA from a male lymphoid cell line, OX. This line was obtained by Epstein–Barr virus transformation of lymphocytes from a patient whose cells contained four Y chromosomes (49,XXXXY)¹⁰. Positive colonies were then transferred to grids and re-screened with a repetitive mouse probe (*Cot*₁₀₀ fraction of total genomic DNA) to eliminate false positives. Approximately 50,000–60,000 colonies were screened in this way and 248 were identified as containing human-derived inserts.

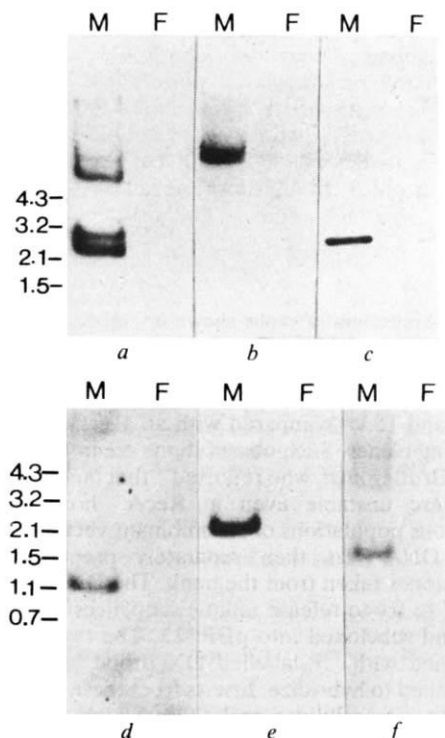


Fig. 1 Hybridization of six essentially single-copy DNA sequences to total human male (M) and female (F) DNA digested with *Eco*RI. Probes show a male-specific reaction pattern. Approximate size is indicated in kilobase pairs. For the preparation of Y chromosome cosmids, high-molecular weight DNA extracted from the 3E7 hybrid cell line was partially restricted with *Mbo*I, size-fractionated on a 10–40% sucrose gradient and the 35–50 kb fraction cloned using vector pJB8 and *E. coli* HB101 (ref. 8). The resulting colonies were screened at low density with the *Cot*₁₀₀ DNA fraction from the human cell line OX, then nick-translated to a specific activity of $1-2 \times 10^8$ c.p.m. μg^{-1} (ref. 16). Hybridization was for 16 h at 65 °C ($2 \times \text{SSC}$) with $30 \mu\text{g ml}^{-1}$ unlabelled sheared BALB/c mouse liver DNA as carrier. Filters were washed four times for 1 h in $2 \times \text{SSC}$ (65 °C) and twice for 10 min in $0.2 \times \text{SSC}$ (20 °C). They were then exposed to Kodak XAR-5 film with intensifying screens at –70 °C for 16 h. Positive colonies were screened with ³²P-labelled *Cot*₁₀₀ BALB/c liver DNA and any cross-reactive colonies discarded. Potential unique-sequence probes were prepared from individual cosmid DNAs, digested with *Eco*RI and subcloned into pBR322. Colonies that failed to hybridize to the ³²P-labelled OX repetitive probe were selected. The plasmids were digested with *Eco*RI, electrophoresed on 1% agarose stained with ethidium bromide and blotted onto nitrocellulose¹⁷. Inserts that again failed to hybridize to the human OX probe were prepared by electroelution from agarose gels and directly nick-translated to an activity of $\sim 0.5-1 \times 10^8$ c.p.m. μg^{-1} . Genomic DNAs for blots were prepared from male and female chronic lymphocytic leukaemia patients or from exponentially growing cell lines (OX). DNA (20 μg per lane) was digested with *Eco*RI, separated by electrophoresis on 0.7% agarose and transferred to nitrocellulose. Hybridization was at 65 °C for 16 h, followed by two washes for 1 h each in $2 \times \text{SSC}$ and two 1-h washes in $0.1 \times \text{SSC}$ (65 °C). Blots were exposed to XAR-5 film at –70 °C for 1–4 days.

To estimate the amounts of material cloned from the heterochromatic portion of Y, the isolated cosmids containing human DNA were screened with the cloned 2.1- and 3.4-kb tandem repetitive sequences which have together been estimated to make up $\sim 50-70\%$ of the Y chromosome^{11,12}. Only 20 of the cosmids were positive with the 3.4-kb probe and one was positive with the 2.1-kb sequence. No cosmids contained both sequences. These data suggest that because of the highly repetitious nature of the fragments they are selected against during the cloning procedure. This would have the effect of enriching the library in sequences derived from the euchromatic region. In fact, all 21 cosmids containing these elements were substantially rearranged, having heterogeneous inserts of

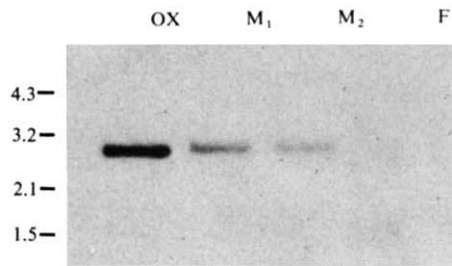


Fig. 2 Hybridization of probe shown in Fig. 1c to DNA from two males (M1 and M2), a female (F) and to DNA from the human cell line, OX, containing four Y chromosomes.

between 5 and 15 kb compared with an average 35–40 kb for the remaining clones. Such observations are in agreement with the data of Brutlag *et al.* who reported¹³ that tandemly repeated sequences are unstable even in RecA⁻ hosts, leading to heterogeneous populations of recombinant vectors.

Cosmid DNA was then separately prepared from 30 individual clones taken from the bank. The DNA was digested with *EcoRI* to try to release unique sequences from repetitive material, and subcloned into pBR322. The resulting colonies were screened with ³²P-labelled OX probe and we selected those that failed to hybridize. Inserts from these subclones were blotted onto nitrocellulose and those which again failed to hybridize to the OX probe were used as potential single-copy sequences. In this way one sequence free of 'middle' repetitive material was derived from each cosmid and used to probe male and female genomic blots restricted with *EcoRI*. As both probes and genomic blots were digested with the same enzyme, recognition of the cognate hybridizing band was possible.

The reaction patterns observed fell into three categories: (1) 6 of the 30 probes were male-specific; (2) four probes showed male–female differential patterns; (3) the remaining 20 probes gave similar patterns with both male and female genomic blots. The six probes exhibiting a male-specific reaction pattern are shown in Fig. 1a–f. Although some of them retain a small amount of residual repetitive material, the male-specific bands are clearly visible. Figure 1c–f detects single bands on the male genomic blots which correspond to the size of the probe used (cognate hybridizing band). Figure 1b detects a larger homologous sequence in addition to the lower cognate band. Similarly, Fig. 1a detects four homologous sequences in addition to the cognate band. An autosomal or X location of these fragments was ruled out as bands were not seen in the female even after prolonged over-exposure. Furthermore, probing equal amounts of DNA from normal males or the OX cell line which contains four Y chromosomes with any of these sequences resulted in a ~3–4-fold increase in signal intensity of the bands on OX DNA, confirming their Y location. Figure 2 shows the result obtained with the probe in Fig. 1c. Finally, the lengths and numbers of hybridizing genomic cognate bands were as predicted by the restriction digests of the original cosmids, implying that these probes consist essentially of single-copy sequences. To our knowledge this is the first demonstration of non-repetitive Y-specific sequences.

The second category of reactivity (male–female differential) is shown in Fig. 3a. This group, comprising four probes, shows one or more bands common to both male and female but also at least one band specific to the male. Again, when OX DNA was probed as in Fig. 3a, both bands increased in intensity (data not shown). This suggests that the bands are localized on the Y chromosome but that the lower cognate band is represented elsewhere in the genome—possibly on the X chromosome. Experiments are in progress to determine its exact location.

The third group of 20 subclones extracted from our Y chromosome cosmid collection—the male–female similar sequences—are shown in Fig. 3b. These probes give between one and four dominant bands in the same position on both male and female DNAs. Three of the probes have so far been

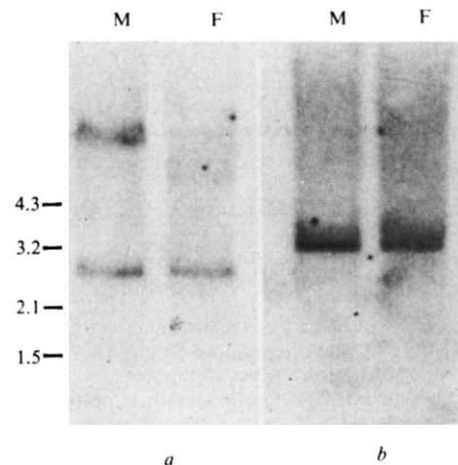


Fig. 3 Hybridization of probes to male (M) and female (F) *EcoRI*-digested DNA. a, Male–female differential reaction pattern; b, a male–female similar pattern of hybridization.

tested on OX genomic blots and in all cases there was an increase in intensity of one or more of the bands. This supports the assumption that such sequences are in fact Y-derived and do not represent sequences cloned from human autosomal or X chromosome fragments which could conceivably be present in undetectable levels in the original 3E7 hybrid cell line. These three subclones also reacted with the mouse–human somatic cell hybrid line, HORL 9X (ref. 14), which contains only the human X chromosome on a mouse background. They may therefore represent common X–Y sequences as described by Page *et al.*¹⁵

The reaction pattern of all three classes of cloned fragments is being characterized further—in particular, the localization of those sequences detected in the female genome by Y-derived probes. The cosmid bank is also being expanded and expressed sequences are being sought. These results will be reported elsewhere.

Our description of Y-derived non-repetitive DNA sequences, including six which react specifically with the human Y chromosome, now allows us to directly probe its organization at the molecular level. Questions such as the postulated exchange of genetic material during X–Y meiotic pairing, conservation of Y-linked sequences during evolution, and the role of the Y chromosome in development, can now be effectively approached.

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Knowledgeable machines

John Fox

Knowledge-based Systems in Artificial Intelligence. By Randall Davis and Douglas B. Lenat.

McGraw-Hill: 1983. Pp.490. \$64.75, £39.75.

KNOWLEDGE engineering is all the rage, just as genetic engineering was a few years ago. There are many similarities between these two new fields. Both have spun off from relatively recent sciences, molecular biology and artificial intelligence. Both attract the attention of the public and of investment managers to an almost embarrassing degree. Both have yet to supply practical products on a large scale.

Fashion is fickle, however, and one might be forgiven for thinking that the knowledge engineering bubble will burst, or that the field will mature in the usual way and be rewarded with a respectable but properly modest place in the scientific establishment. Knowledge engineers, of course, do not believe that this will be the fate of their subject. Indeed, devotees of the parent discipline, artificial intelligence (AI), seem to have higher aspirations than merely "a proper place in the scientific establishment" (though the contributor to a television programme who said that "the three most important events in the history of the Universe were The Creation, the appearance of life, and the discovery of artificial intelligence" must have made even the most flamboyant AI scientist wince a little).

Given such high ambition, I approached the volume by Davis and Lenat with the questions of scientific and engineering credibility in mind. It seemed promising. Both authors did the work described in the book as PhD dissertations at the Stanford University Heuristic Programming Project, currently the leading centre of knowledge engineering. Furthermore the book consists of two complementary monographs; one with a strongly scientific character, one with more of an engineering bias.

Douglas Lenat sees his work on AM (the Automated Mathematician) as scientific research with engineering implications. His efforts are centred on the concept of "discovery", and the extent to which interesting discoveries in number theory can be made using programs that can use simple but powerful rules to generate and evaluate large sets of possible conjectures and hypotheses. For example "if $f(x,y)$ is interesting then $f(x,x)$ may also be interesting". If f is ADD, this suggests the concept of doubling, if TIMES then squaring, and so forth. Lenat gives many examples of more sophisticated "heuristics". The hypothesis that led to AM is that discovery (invention, creativity . . .) should not be assumed to be a mysterious mental faculty, but is the

product of mental information processing mechanisms which are subject to logical analysis and thus to computer simulation. AM produced a few ideas which were new to Lenat, a couple that were "new to the world" and several "... additional 'new' concepts were created which both AM [and Lenat] agreed were better forgotten".

Lenat's work does not show that heuristic search is all there is to human discovery, only that there is a *prima facie* case that computers can be inventive. His hypothesis that discovery could be achieved by a physical device is not new — nothing is — but the demonstration that heuristics can lead to interesting discoveries, now, is dripping with provocative questions.

However, although Lenat clearly feels strongly about the importance of heuristic discovery processes, he is less than clear about the conceptual roots of the method. For example the "interestingness" of a conjecture is crucial to AM's performance, because it controls which of a prodigious set of conjectures will be explored further. Lenat is quite candid that the formula which the program uses to calculate "interestingness" is *ad hoc* — "Since it has worked successfully, I have not messed with it", though he adds subsequently that "... recent experiments tend to confirm that the particular form of the formula is unimportant". Nor does Lenat make it clear what he believes the philosophical status of his achievement to be. He says AM is interesting because it works, but he does not discuss whether heuristic discovery is the (or a) mechanism of human discovery or whether there are likely to be other interesting classes of discovery principle. Here he manifests an engineering reserve rather than a scientific curiosity. An important point I could not find addressed is whether AM is an artifact — successful only because it builds on generations of mathematicians who created the conceptual framework within which the heuristics can be expressed. This need not make Lenat's achievement less interesting but it would perhaps make it less significant.

Much of the current enthusiasm for knowledge engineering has been triggered by rapid growth of the belief that if it is not practical already it will be next week. The appearance of "expert systems" — systems which resemble human experts in carrying out specialized tasks — is largely responsible. One of the earliest and best understood was the MYCIN system which was developed to assist doctors in manage-

ment of bacterial infections. By conventional standards MYCIN is a large program. Much of its size is due to the large body of knowledge it contains about infecting organisms, how they manifest themselves and how they are treated. It appears that the performance of expert systems is determined more by how much knowledge the system has than by the sophistication of the techniques that exploit the knowledge to recommend decisions.

The difficulty in compiling large, comprehensive bodies of knowledge is widely considered to be the main obstacle to commercial exploitation of expert systems. Textbooks are unsuitable and just questioning human experts is not systematic enough. Consequently there is great interest in methods that can speed up the acquisition of knowledge bases. Randall Davis's "Teiresias" grew out of the MYCIN project; it is a program that explores the potential of partially automating the acquisition and refinement of knowledge by interacting with a human expert. Teiresias is named after the blind seer in Sophocles' "Oedipus the King" (*sic*).

Davis distinguishes three types of knowledge. "Object knowledge" refers to the basic application concepts, e.g. medical knowledge of organisms, cultures, drugs etc. "Conceptual knowledge" describes these objects in more general terms; drug dosage is a quantitative concept with values in a particular range, certain organisms have typical features, unique features and so forth. Finally there is "meta-knowledge" or knowledge about how to acquire object and conceptual knowledge from the human expert and represent it in forms that an expert system can reason with.

One aim of the Teiresias project was to refine this idea of meta-knowledge and build an expert system whose expertise is in the construction of other expert systems. Teiresias operates by repeatedly testing the developing expert system on sample problems given by the expert. When the system goes wrong it focuses attention on missing or incorrect information through an elaborate question-answer dialogue. Teiresias helps to focus on the substance of the knowledge that is needed rather than on how the expert system should be programmed, ensuring that the knowledge is fully specified and reminding the expert of all the items that must be supplied and in what form.

A particularly useful section of the book deals with how meta-knowledge can be used to provide explanations of the applications system's reasoning about a problem. This is important for a designer trying to understand why an unfinished system is misbehaving (due to missing or incorrect knowledge). However the facilities are also available in the finalized system and therefore can be used to under-

stand the expert system's recommendations during practical use. This ability to give explanations accounts for much of the interest by software engineers in expert systems' techniques, and Davis gives a clear account of the techniques used in providing explanations, the concepts they are based upon and a straightforward summary of their limitations.

As a whole, the book is an impressive record of two novel pieces of work in artificial intelligence and knowledge engineering. Both monographs provide valuable information for other designers and research workers. Unfortunately, however, the book is not easy to read or easy to use. Minor subjects are dealt with in as great detail as important ones; the text is heavily punctuated with difficult notations and abbreviations; there is little cross-linkage between monographs; and there is no index. The authors are also somewhat inward looking, giving little consideration to previous thinking about knowledge or scientific discovery outside AI, or to the massive body of research on decision support which has not employed knowledge-based concepts. So while their book will be compulsive reading for the committed specialist, I fear that it will fail to convince many people who are trained in other philosophies and traditions.

Davis and Lenat have so much to say that it is a pity that they didn't say it more accessibly. To be fair their work is more briefly written up elsewhere in technical journals, but these are for an informed readership. The book was an opportunity to provide a distillation of profound ideas for a broadly based audience of scientific peers. The authors chose to write a tome. □

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Science and Creationism

BOOKS on the response of scientists to Creationism continue to appear. For the general reader Chris McGowan has written *In the Beginning . . . : A Scientist Shows Why the Creationists are Wrong*, attacking the Creationists while also providing an introduction to the theory of evolution. The book is published by Macmillan of Canada and costs \$18.95.

In similar vein but more detailed is *Scientists Confront Creationism*, edited by Laurie R. Godfrey and published by W.W. Norton. Among the contributors are George Abell, Stephen Brush, Joel Cracraft, Stephen Jay Gould and Thomas Jukes. Price is \$19.50; the book will be published in Britain in October (£15.25).

MIT Press have issued *Creationism, Science, and the Law: The Arkansas Case* which covers the legal and the scientific aspects of the Arkansas case of 1981. The book is edited by Marcel Chotkowski La Follette and is available in hardback (\$20, £18) and paperback (\$9.95, £8.95).

Setting the record straight

Keith Bell

L'écume de la Terre.

By Claude J. Allègre.

Fayard, Paris: 1983. Pp.366. Fr120.

WITHIN a period of less than 20 years, the earth sciences have been turned upside down. Plate tectonics, sea-floor spreading and continental drift now provide a coherent story that links, predicts and explains a wide variety of geological phenomena, from the finding of ore deposits to the understanding of the ocean basins. Few other scientific theories have been so all-encompassing. The evidence that accumulated, partly empirical, partly theoretical and partly analytical, was so overwhelming and so convincing that a somewhat fragmented science was transformed into a smooth, fully-integrated whole. This didn't happen overnight. Ideas developed, stagnated, then were reconsidered, modified and finally accepted.

Behind all this lies a fascinating story that forms the basis of the book by C.J. Allègre, currently director of the Institute of the Physics of the Earth at the University of Paris. Partly philosophical, at times chatty, and never dull, this lucid French-language text traces the development of ideas from Wegener's original model of continental drift through to modern plate tectonic theory. Aimed at a general audience, the book also contains a clearly written and well-illustrated summary of modern geological ideas, complete even with glossary. The gloomy vision of yet another popular tome on plate tectonics and sea-floor spreading was quickly dispelled by the author's fresh approach and his perception of the complex role that the scientific community at large plays in the world of science.

Allègre lays emphasis on an interesting, perhaps often ignored, aspect of science — that the development of any science depends not only upon a scientific discovery itself but also on how it is perceived by the scientific community. We learn, rightly so, that the art of convincing is sometimes more difficult than the art of discovering. That such a book should be written by someone who, no doubt, was soaked in the classical French scientific tradition is, in itself, interesting. The duality proposed in this book of the scientific method on the one hand, and the scientific community on the other, both complementary and both interactive, seems to be a departure from traditional French scientific thought.

Although some stories are left untold, others bring out the tribulations of scientific research, the personalities of the individual researchers and the subtle ways that groups, large and small, interact with

one another. There are one or two factual errors of little consequence, as well as some minor irritations, particularly the naming of contemporary researchers who were only of peripheral importance in the development of the new theories. However, this mixture of a geological *Double Helix* (albeit with a greater cast of characters) and a fluent account of the contemporary earth sciences is absorbing. Because of its unusual philosophical tack this book should be read by anyone interested in scientific endeavour. I hope that an English translation will soon be available. □

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The haemoflagellates

F.E.G. Cox

The Biology of Trypanosoma and Leishmania, Parasites of Man and Domestic Animals.

By D.H. Molyneux and R.W. Ashford.

Taylor & Francis: 1983. Pp.294.

£16, \$36.

THE haemoflagellates *Trypanosoma* and *Leishmania* spp. cause Chagas' disease, sleeping sickness and leishmaniasis in man, and nagana and other diseases mainly in cattle and horses. These parasites have, over many years, attracted a vast amount of attention and the aim of the authors of *The Biology of Trypanosoma and Leishmania* is "to bridge the gap between what appears in textbooks and the appropriate research texts". They have tackled this formidable task remarkably successfully.

The first part of the book deals with the essential biology of the parasites and the tsetse flies, triatomid bugs and sandflies that transmit them. This section is not simply descriptive, but covers such diverse topics as ultrastructure, isoenzyme techniques in the identification of species and strains, antigenic variation, insect behaviour and insect trapping methods. The second part is concerned with African trypanosomiasis (both human and animal) and Chagas' disease, and the third with leishmaniasis. These three sections touch upon all the important facets of the infections caused by these parasites, including clinical features, treatment and control. There are two useful appendices summarizing some basic information about trypanosomes that are not of economic importance, those of small mammals, birds, reptiles and amphibians, and *Leishmania enriettii*, *L. hertigi* and *L. gerbilli*. Widely distributed parasites such as *T. theileri* are mentioned throughout the book.

This summary of the contents, however, does this timely and invaluable book scant justice. Throughout, the emphasis is on

the biology of the organisms. Details of biochemistry, immunology and pathology are kept to a minimum, but enough information is given to inform the reader about various important facts without impeding the logical flow of the text. The style is buoyant and refreshing, and the book could be read from beginning to end with enjoyment. It is fully and reasonably well illustrated although not all the photographs are of the best quality. This is a volume that should be bought by all those working with haemoflagellates, their associated diseases or vectors, and by those taking postgraduate or even undergraduate courses in parasitology because it contains a vast amount of information not available in such an accessible form elsewhere.

There must, of course, be criticisms of a book of this kind and some readers, particularly those in South America, may feel that *T. cruzi* has been given a disproportionately small share of the text; be this as it may even those readers will learn something from the rest of the book. The references, however, are inadequate. There are less than 70, grouped together at the end of the book without extensive cross-referencing between the text and the bibliography. Similarly, there is nothing to link the figures directly to their sources. These defects are likely to be particularly infuriating if the book is to be used as a bridge between textbook and research paper, but there will undoubtedly be further editions and such problems can be attended to then. □

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All around reactors

Malcolm C. Scott

Nuclear Fission Reactors.

By I.R. Cameron.

Plenum: 1983. Pp.390. \$42.50, £29.75.

THE nuclear industry is now well established, therefore requiring a supply of trained staff. Its growth is also contentious, making well-informed protagonists and antagonists essential if energy investment programmes are to be other than political slanging matches. Yet, despite these two factors, there have been relatively few books which have provided the fundamental introduction and the breadth required to suit both aspiring specialists and those wanting a more general background.

For this reason alone J.R. Cameron's *Nuclear Fission Reactors* must be welcomed. In addition, the author has a fluency of style and a clarity of presentation which makes the book a pleasure to read. Lest "pleasure" implies a lightweight work, let me hasten to add that part of the pleasure derives from seeing how the

author manages to introduce a mass of detailed scientific and technical ideas, and interrelate them, and yet retain a lightness of touch which makes it all seem easy to understand.

Although the book stands as an integrated whole, giving a sound grounding in the physical and technological basis for nuclear power, Cameron has broken the subject down into four sections. The first, of three chapters, covers the physics of the atom and nucleus and then builds up, through a description of fission and the chain reaction, to an account of nuclear reactor theory, based on the diffusion approximation. Up to this point the ground is well trodden, though this is a particularly clear exposition, with worked examples to assist absorption.

The next three chapters are also general in scope. The first two cover the materials' aspects of nuclear fuel and other in-core materials. There are sections on fuel enrichment, and the importance of radiation damage is emphasized in the descriptions of individual materials. The next chapter, which concludes the general section, examines heat transfer and the steam cycle.

After a brief introduction, the third part of the book consists of four chapters, one on each of the main reactor types. Perhaps because of his background (one deduces that Cameron is an Englishman who has emigrated to Canada) the author has resisted the temptation to cover light-water reactors in detail to the exclusion of other systems. Instead we have roughly equal weight given to fast reactors, light-water, heavy-water and gas-cooled systems. Finally, a single chapter (the longest) considers safety and environmental effects, incorporating — inevitably and necessarily — a section on reactor accidents which includes an account of events at Three Mile Island. With characteristic thoroughness the author starts this chapter with several pages on the biological effects of radiation and finishes with a section on the long-term waste-disposal problem.

In a book of this breadth, individual specialists will find areas where emphases are felt to be misplaced or where information is, perhaps, a little dated. Additionally, a description of some of the reactor systems operating, or under consideration, in the USSR would have been interesting whilst some readers may be off put by the use of imperial units (surely an SI version will appear soon?). But these are minor quibbles. This is a roundly conceived and well-written book — still all too rare outside mainline undergraduate texts — which provides a superb introduction to nuclear reactors for the non-specialist and aspiring specialist alike.

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Stevenson's humus

M.H.B. Hayes

Humus Chemistry: Genesis, Composition, Reactions.

By F.J. Stevenson.

Wiley: 1982. Pp.443. £37.95, \$53.15.

HUMUS, the product of biological and chemical transformations of plant and animal residues, is present in all soils and waters. In the soil environment humus substances stabilize soil aggregates, act as reservoirs for water and cations, and provide substrates for the teeming soil biota. After mineralization they yield valuable reserves of N, P, S and trace elements for plant growth. Less desirably, some humus components can inactivate soil-applied organic chemicals, and certain fragments of these components, with or without associated applied chemicals, could present health hazards when transported into waterways.

Thirty years ago F.J. Stevenson made an outstanding contribution to our understanding of the electrokinetic properties of humic acids. Since that time he has been at the forefront of research in several other areas of humus chemistry, and there are few people better qualified to prepare a comprehensive treatise on the subject. His new book comes up to expectations almost fully.

Here Stevenson provides all of the pertinent quantitative background to humus materials, and he discusses their environmental significance in detail. His treatment of the genesis of humus is, in my view, the best available. Similarly his coverage of extraction and fractionation procedures, and the treatment of soil saccharides, organic nitrogen, phosphorus, sulphur and lipids in humus is clear and to the point. Interpretations of studies of the surface and colloidal properties of the polymers, and of their binding of heavy metals are outstanding.

In view of the importance of humus materials, both in soils and water, it is perhaps surprising that so little is known of their structures. Stevenson rightly points out that a better knowledge of their formation could help overcome this deficiency. The difficulty lies in the intractable yet intriguing chemical make-up of the polymers in which the labile peptide bonds and glycosidic linkages are peripheral to the core structures. These structures are held together by difficult to cleave carbon-carbon bonds and ether linkages, and so it is hard to degrade humic materials to products which can be related to primary structures. Because their synthesis is not genetically controlled, it is possible that very few molecules are similar in any batch, and hence attempts at determining secondary structures would be meaningless. The information which is available about tertiary structures is considerably better,

however. Humic acids, soluble in buffers at pH 9, are highly polydisperse and take up random coil conformations in solution. Branching increases with molecular weight, and it is probable that the higher molecular weight, less-soluble components are highly branched and/or cross linked. This information allows some, albeit incomplete predictions to be made about the shapes of the polymers in the solid or gel phases important in the soil environment.

I was a little disappointed by Stevenson's approach to studies of humic structures through degradative procedures. Although he recognizes the pitfalls which have hindered interpretations of polymer structures from digest products, he does not stress these pitfalls enough or give sufficient examples of the needs for model studies which show the extents to which these products are artifacts formed in the reactive digest conditions. Since the manuscript was finished, several exciting applications of CPMAS ^{13}C NMR have helped our understanding of differences between humic substances from different origins, especially with regard to variations in aromatic-aliphatic ratios in the polymers. Now the combination of degradation procedures and modern spectroscopy promises to provide exciting new information about humic structures.

Humus Chemistry is compulsive reading for every serious student of soil organic matter-humus, and of humic substances. The chemical approach to studies of these substances is rarely emphasized in teaching, but with the appearance of this book there is no longer any excuse for the teacher who has found it difficult to grasp the principles of the subject. □

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Quantum mechanics in the cold

A.J. Berlinsky

Solid Hydrogen: Theory of the Properties of Solid H_2 , HD, and D_2 .

By Jan Van Kranendonk.

Plenum: 1983. Pp.306. \$39.50, £27.65.

IN PRINCIPAL, the solid hydrogens, H_2 , HD and D_2 , are the simplest of all molecular solids, resembling light, rare-gas crystals with extra degrees of freedom corresponding to vibration and rotation of the internuclear axes. It so happens, however, that a number of important quantum mechanical effects conspire to make these solids not so simple. The result is an amazingly rich variety of solid state phenomena.

Hydrogen freezes at 14 K and D_2 at 18 K. One remarkable property of these solids is that, even at such low temperatures and in fact down to $T=0$, the molecules act as free rotors. That is, the rotational states of the molecule are hardly perturbed from what they would be in the gas. The rotational levels are given by $E_J = BJ(J+1)$ where J is the rotational quantum number and B , which is 85 K for H_2 and half that for D_2 , is very large, compared to its value in heavier molecules and to the temperature of the solid.

Since the energy of the $J=1$ level is large compared to kT , one would expect all molecules to be in the $J=0$ state in the solid. This is true for HD. However, for H_2 and D_2 , quantum mechanics intrudes again, via the Pauli principle, which for H_2 requires that total nuclear spin $I=1$ states must combine with odd rotational states, the result being called ortho-hydrogen, and

the nuclear spin $I=0$ state combines with even J giving para- H_2 . At low temperatures, para- H_2 molecules are essentially all in the low-energy $J=0$ state while ortho- H_2 molecules are predominantly in the $J=1$ state, which is metastable because the combined transition ($J=1, I=1 \rightarrow J=0, I=0$) is strongly forbidden. Analogous arguments apply to solid D_2 .

The picture which emerges is that solid H_2 and D_2 are actually alloys of $J=0$ molecules whose orientational wave functions look like spheres and $J=1$ molecules whose wave functions look like electronic p -orbitals. $J=0$ molecules are orientationally inert, acting very much like rare gas molecules, while $J=1$ molecules can interact, mainly via electric quadrupole-quadrupole interactions, and this interaction energy depends on the relative orientation of the wave functions of interacting pairs of molecules. Physicists have developed methods for separating ortho- and para-hydrogen, and the result is that experiments can be done on crystals containing $J=1$ concentrations ranging from 0.02% all the way up to 99.8%. The great variety of phenomena which result are analysed in a detailed and systematic way in Jan Van Kranendonk's *Solid Hydrogen*.

The book is the work of a theorist who has calculated many of the experimentally observed properties of solid hydrogen. The emphasis is on the spectroscopy of the solid by Raman scattering, infrared and microwave absorption, and nuclear magnetic resonance. A sample of the topics treated includes the exciton-like behaviour of $J=2$ excitations in a $J=0$ solid; the lattice dynamics of quantum crystals, with special emphasis on the way that intermolecular interactions are renormalized by the large amplitude zero point motion; and properties of small clusters of $J=1$ molecules in the $J=0$ solid, particularly pairs of $J=1$ molecules, which exhibit a rich and well-resolved microwave spectrum.

Also included is a discussion of the pure $J=1$ solid, where the rotational wave functions form an orientationally ordered state at low T which is closely analogous to an antiferromagnetic state. Excitations of this state are angular momentum waves which are called librins. The final chapter, on ortho-para conversion, also describes a distinctive type of quantum diffusion in which a $J=1$ and $J=0$ molecule exchange positions by exchanging their identities.

The overall emphasis of the book is on the theoretical analysis of these phenomena. It is certainly not a handbook of the measured properties of solid hydrogen. On the other hand, for those who are interested in how one calculates the properties of the solid hydrogens, it will be a very useful reference book indeed. □

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Close-up on the natural world — vertical section of an unripe fruiting head of a dandelion, *Taraxacum officinale* (x27). The picture is taken from D. Claugher's *Scanning Nature*, a superb collection of scanning electron micrographs recently published by the British Museum (Natural History) with Cambridge University Press. Prices are hbk £13.50, \$22.50; pbk £5.50, \$9.95.

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